COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

THE NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY

A. AGRICULTURAL RESEARCH SECTION

VOLUME 31.—No. 1 JUNE, 1949

Printed for the Department of Scientific and Industrial Research, Wellington,
by
Coulls Somerville Wilkie Ltd., Dunedin, N.Z.
July, 1950

REGISTERED AT G.P.O., WELLINGTON, AS A MAGAZINE

9 JAN. 1951

THE NEW ZEALAND

Journal of Science and Technology

VOLUME 31, No. 1, 1949

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SUBSCRIPTIONS.—Subscriptions payable in advance. Series A, 10s. per annum; Series B, 10s. per annum; A and B, 15s. per annum. Single copies, 2s.; postage, 1d. Subscriptions should be paid by money-order, postal note, bank note or cheque, and should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 562, Dunedin. Cheques should be made payable to the Department of Scientific and Industrial Research, and when payable at banks other than in Dunedin should have exchange (6d.) added. Subscribers are requested to furnish their full names and addresses.

CORRESPONDENCE.—All communications relative to the Journal should be addressed to the Editor, Department of Scientific and Industrial Research, Wellington.

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

THE NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY

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THE NEW ZEALAND
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Editor: N. A. Marris, M.Sc., B.Com. (Assistant Editor: M. O'Connor, M.Sc.)
Department of Scientific and Industrial Research, Wellington

VOL. 31, SEC. A JUNE, 1949 NUMBER 1

THE EFFECT OF STARVATION, AND OF FEEDING AFTER STARVATION, ON METABOLIC ACTIVITY IN THE RUMEN

By I. E. COOP, Canterbury Agricultural College, Lincoln, New Zealand

(*Received for publication, 16th June, 1949*)

Summary

A sheep with a rumen fistula has been starved for periods of three to four days and then fed. Changes in the pH and volatile acid content of the rumen have been measured. A measure of microfloral activity has been obtained by determining, *in vitro*, the rate at which ruminal samples ferment glucose and lotaustralin. It has been shown that during starvation microfloral activity rapidly decreases to a low level. When the sheep is fed after starvation, the recovery to normal ruminal activity takes from 12 - 24 hours. The trends in pH and volatile acid content of the rumen support these observations.

INTRODUCTION

SHEEP and cattle in most stock raising countries are sometimes forced to undergo starvation for a period of several days. This occurs most frequently when stock are sold or bought through saleyards. Usually no food or water is provided for the stock from the time they leave the vendor's farm till they arrive at the purchaser's farm. Where the transport is made by rail it may involve railage to and from the saleyards together with six to twelve hours in the saleyards. At either end of this period there may be some droving, yarding, and drafting. It is therefore not uncommon for sheep and cattle to go for two days and in certain cases for three or four days without either food or water.

Provided the stock are in reasonable condition and health such treatment is relatively harmless. But experience has shown that when the sheep so treated are turned on to lush pasture or on to grazing containing toxic plants the effect is sometimes far from harmless. The hungry stock may gorge themselves, thus upsetting the normal processes of digestion or, in the case of toxic plants, may eat sufficient to provide a lethal dose. Franklin and Reid (1) in Australia reported mortality from feeding linseed nuts to hungry sheep after a long rail journey. Coop and Blakley (2) showed that the rate of release of hydrocyanic acid from cyanogenetic plants, and hence the toxicity of the plants, may be affected by starvation of the animal.

It is probable that in these and other instances, conditions within the rumen have a profound effect. It was decided therefore to measure the effect of starvation and of feeding after starvation, on the metabolic activity occurring in the rumen. This activity, resulting mainly from

the bacteria and other micro-organisms present, is influenced by a number of factors such as the nature of the previous diet, the rate of food and water intake, and the pH. Furthermore, the effects produced are undoubtedly varied and may consist of changes in absolute concentration, in relative proportions of the various types, and in the metabolic rate of individual micro-organisms. The difficulties of measuring separately the various causes and effects are considerable. Attempts have been made by identifying and counting the various types of micro-organisms to determine the effects of feeding. Thus Johnson *et al.* (3) and Bortree *et al.* (4) showed that after feeding there were rapid increases in the concentrations of certain bacteria. Changes in the relative proportions of the different types of microflora following food intake have also been reported by Quin (5), Van der Wath (6) and Johnson *et al.* (3). Unfortunately, with the present techniques available it is not possible to identify and count all types of ruminal micro-organisms, though the changes recorded above may justifiably be taken as characteristic.

Another approach is to measure the summation of all effects, namely, the over-all metabolic rate. This can be done by determining the rate at which end products are produced from some added substrate by a sample of ruminal fluid. Quin (5) and Jacobson *et al.* (7) used gas production, while Phillipson (8) used volatile fatty acid production as an index of metabolic activity in the rumen. Quin (5) showed that after feeding lucerne to a sheep there was a rapid increase in gas production reaching a peak within 30 minutes and then an almost equally rapid decrease in production. Small variations in rate of gas production were recorded between different sheep on the same diet, the same sheep on different diets and the same sheep at different times of the day. Starvation for two to three days caused a large decrease in rate of gas production from glucose and the original activity was not fully recovered until ten days after feeding again. Phillipson (8) found that rate of volatile acid production in the rumen depended on the diet. Greatest activity was observed when the diet was high in soluble carbohydrate and least when the diet was fibrous.

Coop and Blakley (2) found that the rate at which HCN was released by ruminal microflora from the cyanogenetic glucoside, lotaustralin, varied from day to day. They also found that when a normal sheep was dosed with lotaustralin, cyanide poisoning developed rapidly, but when a sheep was starved for several days, then dosed and given food, no symptoms occurred for several hours, after which they developed rapidly. This observation was interpreted as showing that in the starved sheep metabolic activity is at a low level so that the lotaustralin was not hydrolysed. However, when food was given the microfloral population increased and the medium for metabolic activity improved with increasing momentum to the point where the lotaustralin was suddenly hydrolysed some hours after dosing.

In the present work a measure of the overall metabolic activity was obtained from the rate of volatile fatty acid production from glucose, and of free HCN from lotaustralin. Such a criterion ignores the individual effects of microfloral concentration and proportion but measures the combination of all factors. It is realized that the two substrates chosen are in practice not quantitatively important in the diet of ruminants, but this objection is reduced by the fact that probably a large variety of organisms can utilize both glucose and lotaustralin. The object of using lotaustralin as a substrate was simply that the reaction

is much faster and easier to measure than the glucose reaction. The simple kinetics of microfloral enzymic reactions are open to the criticism that the micro-organisms may multiply during the time taken to measure rates of reaction. To minimize this the initial rate up to a maximum of 50 per cent hydrolysis of the substrate was measured. From the shape of the velocity—time curve obtained, it was evident that, with the incubation technique used, there could have been little increase in concentration of the micro-organisms during the reaction *in vitro*.

EXPERIMENTAL METHODS

The sheep used for the experiments was a four year old Romney wether with a permanent rumen fistula. At the time of these experiments it weighed 70 - 80 kg. and ate readily up to 3 kg. of lucerne chaff per day.

The pH of ruminal samples was measured by a glass electrode pH meter within ten minutes of taking the sample from the sheep.

Volatile Acid Production from Glucose

The technique of fermentation and volatile acid production was essentially the same as that used by Elsden (9). The incubation medium comprised 35 ml. of buffer, 5 ml. of 10 per cent glucose and 10 ml. of ruminal liquor. The buffer maintains the pH near neutrality and hence observed rates of reaction apply to the pH of the medium and not to the original pH of the rumen. Volatile acid was determined by Friedemann's method as modified by McAnally (10) and was calculated as acetic acid. Allowance for initial volatile acid present and that developed in the ruminal liquor alone, was made by incubating a blank without glucose. Volatile acid production was plotted against time and the time taken for 50 per cent production (a titre of 7.5 ml. of 0.01 N KOH per ml. of incubation fluid) was estimated. This is recorded as the half time. The metabolic activity towards glucose at the pH of the buffer is then inversely proportional to the half-time.

Increase of glucose concentration increased the rate of volatile acid production slightly but when the concentration in the medium was raised to four per cent inhibition took place. Dilution of the ruminal fluid decreased the rate of reaction approximately, though not strictly, in proportion to the dilution. Under normal feeding conditions the half-time was of the order of six to eight hours but when the ruminal fluid was diluted or after a period of starvation, the half-time became of the order of 30 hours. In such cases it is possible that the microfloral concentrations change significantly. Although Elsden (9) considers that in the first 24 hours this is not serious, the half-time was determined in these cases by extrapolation from observations within the first 12 - 24 hours. From errors in duplicate estimations and from dilution experiments, it is estimated that the errors in determining the half-time are of the order of $\pm$ 10 per cent for half-time less than ten hours, and $\pm$ 20 per cent for half-times in the range 20 - 40 hours.

The breakdown of glucose to volatile acids can under certain circumstances proceed via lactic acid. It was shown in the present work that the production of volatile acids proceeds as rapidly from glucose as from the same concentration (w/v) of lactate.

Hydrolysis of Lotaustralin

The hydrolysis of lotaustralin by ruminal microflora and the method of determination of HCN have been described by Coop and Blakley (2). Before adding the lotaustralin to the ruminal fluid the pH of the latter was brought by addition of acid or alkali within the range 6.0 - 7.2. Corrections were then made for this dilution in the calculated velocity. A further correction was also made relating the actual pH to the optimum, 6.8, according to the pH-activity curve given by Coop and Blakley. The rates of hydrolysis or half-times quoted are therefore those at a pH of 6.8 and not at the pH of the sample as taken from the rumen.

Under normal feeding conditions the half-time is of the order of five to ten minutes. Even when the half-time is increased 100 times by dilution or starvation the time available for deviation from the initial microfloral concentrations is much less than for glucose. The estimated errors in determination of the half-time for lotaustralin are ± 10 per cent for all times.

RESULTS

Normal Feeding

The variation in activity when a sheep is fed normally, i.e. with food and water available all the time, was measured over a period of several days. The food given was chaffed lucerne hay. Records of food and water consumption were kept to determine whether any correlation could be made between the variation in activity and the food or water intake.

The results of this experiment are set out in Fig. 1.

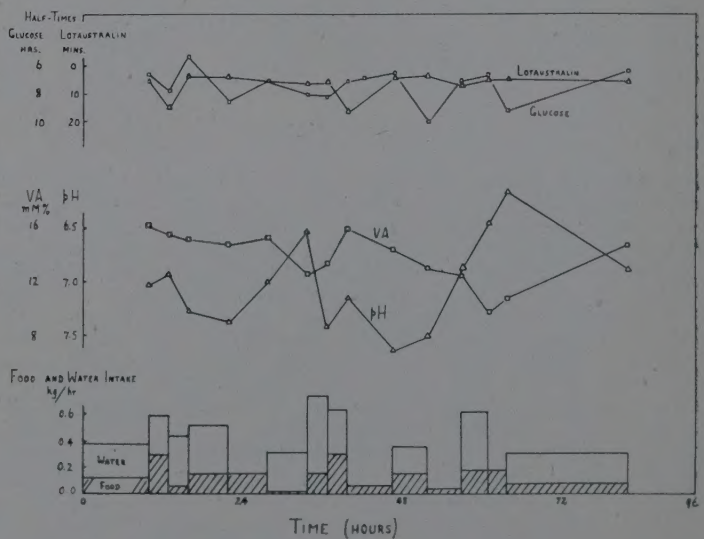

FIG. 1.—Variations in pH and volatile acid content of rumen and in activity of ruminal micro-organisms towards glucose and lotaustralin in a sheep eating normally.

It will be seen that the activity of the microflora remained at a high level throughout the period, the half-times for glucose and lotaustralin being within the range five to ten hours and three to eighteen minutes respectively. There was no correlation between pH of the rumen and the volatile acid content, nor between the activity for glucose and for lotaustralin. In addition there is no consistent variation attributable to the food or water intake over the periods between sampling.

This experiment was repeated, taking samples at more frequent intervals ($\frac{3}{4}$-hour intervals) as it was thought that any effect of food and water intake could have been missed in the previous experiment if sampling was not done immediately after food or water intake. Again no consistent correlations of any kind could be observed. Even introducing two to three litres of water directly into the rumen and allowing half an hour for complete mixing made very little difference at the end of the half-hour, an observation in agreement with Phillipson (8).

Starvation and Recovery from Starvation

Trial I (25 - 28 July):

The sheep which had been eating lucerne chaff at an average rate of $2\frac{1}{2}$ kg. per day before starvation began was deprived of both food and water for three days. At the end of this time the rumen was almost empty, and what material was present was moderately fluid. When offered lucerne chaff again it ate 0.10 kg. in the first hour but then ceased eating. At + 3 hours it was given fresh green grass of which it ate 0.9 kg. between + 3 and + 5 hours, 0.35 kg. from + 5 to + 7 hours and 0.4 kg. from + 7 to + 24 hours.

The measurements made are recorded in Table I.

TABLE I.

Time. (hr.)	pH.	V.A. (mM. per cent.)	HT (Glucose). (hr.)	HT (Lot.). (min.)
−72	6·45	10·2	8	13
−48	7·20	5·8	20	45
−24	7·40	5·1	38	240
0	7·56	4·8	50	660
+ 3	7·40	5·7	—	117
+ 5	6·64	9·0	20	—
+ 7	6·38	11·3	17	53
+12	6·82	12·4	17	—
+24	7·28	11·4	9	39

NOTE: Time. Zero time is the time feeding commenced after starvation. (*e.g.*,–72 hours is the commencement of a 72 hours starvation period).
pH. pH of rumen.
V.A. Volatile acid concentration in the rumen in milli-moles (mM.) per cent.
HT (Glucose). Half-time for glucose in hours.
HT (Lot.). Half-time for lotaustralian in minutes.

It will be seen that the activity towards both glucose and lotaustralin fell to a very low level, the lowest recorded at any time in these experiments. Recovery to normal activity was probably of the order of 12 hours though the data is not complete.

Trial II (13 - 17 August):

The sheep was brought into pens from pasture, starved of both food and water for three days, and then turned back on to pasture. At the end of the three days there was a considerable amount of material in a fairly solid state still in the rumen, which may account for the fact that the activity towards glucose and lotaustralin was not reduced to a particularly low level.

On turning out to grass no record of food intake could be made, but from the appearance of the ruminal samples it was deduced that the sheep drank heavily and ate only modest amounts of grass. The results are recorded in Table II.

TABLE II.

Time. (hr.)	pH.	V.A. (mM. per cent.)	HT (Glucose). (hr.)	HT (Lot.). (min.)
−72	6·14	15·2	6½	8
−48	7·51	5·5	16	16
−24	7·62	4·6	—	24
0	7·58	5·6	26	32
+ 2	6·80	8·6	20	65
+ 4	6·49	11·6	12	52
+ 6	6·32	14·8	8	22
+ 9	5·73	19·5	5	5
+12	5·71	20·7	3½	4
+24	5·81	18·3	—	5

Apart from the values of activity towards lotaustralin at 0, + 2 and + 4 hours there was remarkable agreement between the activities towards glucose and lotaustralin and between pH and V.A. measurements. When plotted these results very closely resemble those to be given in Fig. 2. By + 9 hours the activity had surpassed that before starvation, and reached a peak at between + 12 and + 24 hours.

Trial III (26 - 31 August):

The trial followed on immediately after a period of normal feeding on chaffed lucerne hay as illustrated in Fig. 1. The sheep was starved of food and water for four days and then given lucerne hay and water again. Records of food and water consumption were noted. The results of this trial are plotted in Fig. 2. They are typical of all the three to four day starvation trials showing the effect of starvation and the recovery to normal, the latter occurring in 18 hours.

The amount of food and water consumed by the sheep during the trial was as follows:—

Feeding Period. (hr.)	Material Consumed.	
	Hay. (kg.)	Water (l.)
0 to + 4	0·6	
+ 4 to + 6	0·2	1·2
+ 6 to + 8	0·2	0·7
+ 8 to +10	0·2	0·5
+10 to +14	0·3	—
+14 to +29	0·75	3

The volume of ruminal contents had returned to normal by + 14 hours.

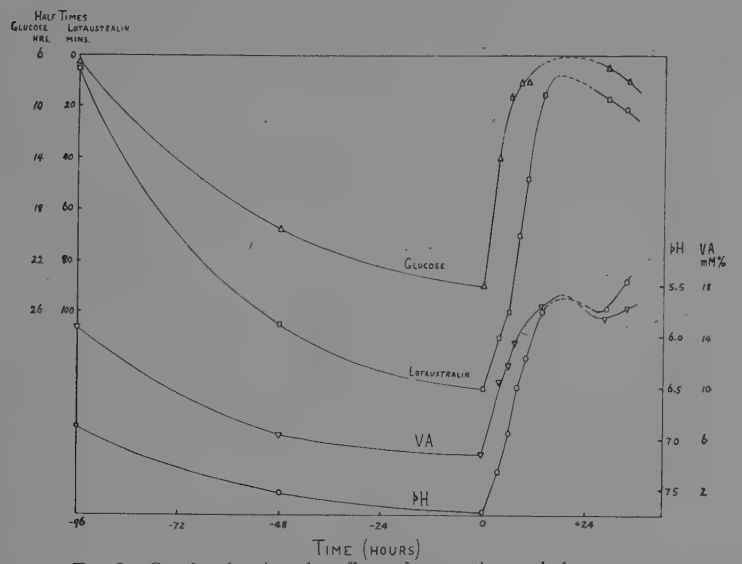

Fig. 2.—Graphs showing the effect of starvation and the recovery from starvation, in respect to pH and volatile acid content of the rumen and to the activity of ruminal micro-organisms towards glucose and lotaustralin.

Trial IV (3 - 8 September):

The sheep after feeding on lucerne hay was starved for three days and then given chou moellier. Results are recorded in Table III.

Table III.

Time. (hr.)	pH.	V.A. (mM. per cent.)	HT (Glucose). (hr.)	HT (Lot.). (min.)
−78	7·23	8·3	8	11
0	7·92	4·4	32	200
+11½	7·19	8·7	24	26
+13½	7·15	9·5	20	28
+15¾	6·95	9·4	17	13
+18½	6·80	10·8	12	7½
+22	7·46	9·8	14	16

They follow the same pattern as that illustrated by Fig. 2. The time taken to recover to normal ruminal activity was 20 hours as measured by peak activity in the period after starvation.

During the period of this trial the sheep consumed the following amounts of food and water:—

Feeding Period. (hr.)	Material Consumed. Choumoellier. (kg.)	Water. (l.)
0 to +11½	1·6	—
+11½ to +13½	—	—
+13½ to +15¾	0·45	0·3
+15¾ to +18½	0·7	0·1
+18½ to +22	—	—

Trial V (20 - 22 September):

In this case the sheep was starved for one and a half days only, being taken off grass and returned to grass. The usual pattern occurred, recovery taking about 12 - 16 hours. The measurements taken are recorded in Table IV.

TABLE IV.

Time. (hr.)	pH.	V.A. (mM. per cent.)	HT (Glucose). (hr.)	HT (Lot.). (min.)
−34	6·68	13·8	2	4
−30	6·61	12·7	—	6
− 9	8·32	4·1	6¾	12½
0	8·11	3·8	18	32
+12	6·10	16·2	5½	6½
+15	6·22	16·6	—	3
+17	6·06	18·3	4	6½
+20	6·11	17·5	3½	4

Recovery in vitro

The course of the lotaustralin hydrolysis was followed *in vitro* to determine the effect of adding glucose or dried grass to ruminal fluid. After starving the sheep for three days samples of ruminal fluid were taken and incubated as follows:—

(i) 10 ml. ruminal fluid + 1.0 ml. of lotaustralin.
(ii) 10 „ „ „ + 1 ml. of 10 per cent glucose.
(iii) 10 „ „ „ + 0.25 g. dried rye-grass (free from HCN.)

The results obtained are plotted in Fig. 3.

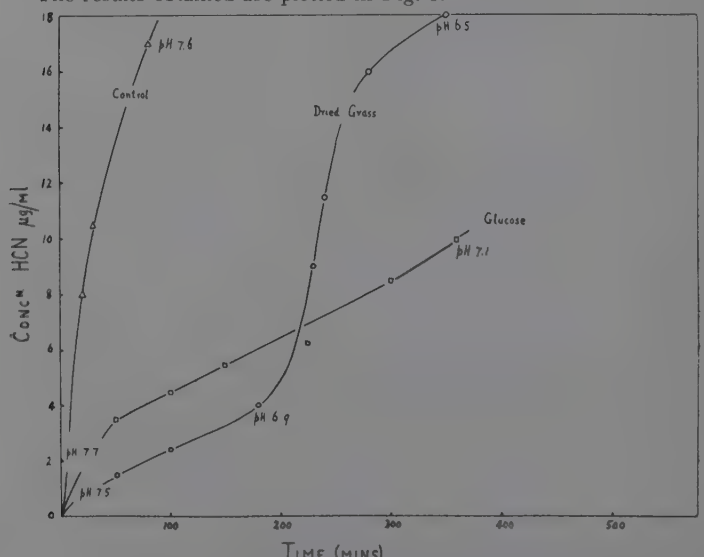

FIG. 3.—Effect of addition of glucose or dried grass on rate of hydrolysis of lotaustralin. (Complete hydrolysis is equivalent to 27 µg HCN/ml.)

It will be observed that the glucose produced a strong inhibition. The grass inhibited even more strongly, but after 200 minutes the rate increased very rapidly to the uninhibited value. This is presumably due to: (a) decrease in pH to near the optimum value (pH 6.8); (b) multiplication of micro-organisms induced by the added grass during the 200 minutes; and (c) the readily available nutrients provided by the grass and competitively inhibiting the reaction having been metabolized by the end of approximately 200 minutes.

The experiment was repeated and essentially similar results obtained. The glucose concentration was altered to 1 ml. of 5 per cent and 1 ml. of 20 per cent. The degree of inhibition was respectively decreased and increased in comparison with the previous experiment but in neither case was any steep upward trend observed. Decreasing the dry grass to 0.15 g. did not produce sufficient inhibition to cause the phenomenon to occur, whilst increasing it to 0.45 g. produced so much inhibition that the upward trend was much reduced. It appears from this that there are optimum conditions in respect to the amount of dry grass added to produce inhibition followed by acceleration.

Discussion

If a sheep were to graze continuously one would anticipate that in the rumen a stable condition would be reached, in which microfloral concentration and activity would be fairly constant and in which rate of volatile acid production and absorption would be approximately equal. As the food and water intake of a sheep feeding or grazing naturally is not regular, fluctuations in the pH, volatile acid content and microfloral activity would occur in practice. The more irregular the intake the greater would be the fluctuations.

As far as pH and volatile acids content are concerned, one would expect to find an inverse relationship, since acidity in the rumen is due to the volatile acids together with lactic acid, the latter being a transient intermediary product. In a broad sense this inverse relationship is true, as shown by Phillipson (8) and in the present work. When the pH is near 6.0 the volatile acid content is high and when near 8.0 the volatile acid content is low. This relationship, however, does not hold in detail. The results with a sheep feeding normally presented in Fig. 1 show that the short-term or small-magnitude fluctuations in pH bear no obvious relationship to the volatile acid content. The secretion of saliva, dilution of ruminal contents by the food and water intake, and the pH of the food eaten would all tend to upset any exact relationship. It must be concluded therefore that on the diet used (lucerne hay) the pH of the rumen is, except in a general sense, a poor index of volatile acid content. Other diets, containing different amounts of readily fermentable carbohydrate or giving a different proportion of lactic acid production may give a different result.

On the other hand the pH and volatile acid content of the rumen do remain within reasonable limits in the sheep feeding normally. Phillipson showed with sheep fed in the morning after an overnight fast, that minimum pH values and peak volatile acid concentrations were reached about four hours after feeding a diet rich in readily available carbohydrate, and about 12 hours after feeding a fibrous diet. Similar results have been obtained by Iggo (11). These times are long in comparison

with normal intervals of feeding. It is also probable that the time taken for a given intake of food to cause a multiplication of micro-organisms by virtue of providing the medium for growth, is greater than the intervals between periods of food intake. These two factors would tend to smooth out any large fluctuations due to variations in rate of food intake. In the case of microfloral activity the half times for glucose and lotaustralin were within the range five to ten hours and three to eighteen minutes respectively on all occasions when the sheep was feeding normally. The fluctuations observed were relatively small. Nevertheless, considering the number and complexity of the factors influencing conditions in the rumen, it is not surprising that as shown in Fig. 1 no relationship could be traced between food intake and any of the other things measured—microfloral activity, pH and volatile acid content.

Much clearer and more consistent relationships can be found when sheep are starved. The effect of starvation on the ruminal contents is firstly to reduce the volume from a normal value of three to four litres down to half to one litre after three to four days. A more important effect is the reduction in microfloral concentration. After three to four days of starvation (removal of both food and water) the ruminal contents may not necessarily be dry but most often contain pale fibrous particles in a pale transparent orange-brown fluid. The latter, when examined microscopically, is seen to contain relatively few micro-organisms. On the other hand when ruminal activity is at its highest, the fluid has the appearance of a thick greenish-grey sludge with a very high concentration of micro-organisms. No microfloral counts were made in the present work, but when samples were examined microscopically an obvious relationship between activity and microfloral concentration was apparent.

In starvation, variations due to food and water intake are eliminated and this leads to greater consistency between the supposedly related measurements. Consistent trends become easily discernible. The starvation experiments show that the microfloral activity and volatile acid content fall rapidly for the first one to two days and thereafter fall slowly. If the true activity as represented by the inverse of the half-time is plotted, the decrease in activity during the first day is emphasized much more strongly than when the half-times as recorded in the Tables and Figures are plotted. The actual decrease in the first day is complicated slightly by the amount of food eaten in the few hours preceding removal of food and water. If a large intake has occurred the decrease is much less marked. It will be seen from the Tables and Figures given that three to four days of starvation reduces the volatile acid content from a normal value of 10-20 mM per cent down to 4-6 mM per cent. This latter level is not particularly low, presumably because of the high pH causing most of the acid to be in the ionic form. It will also now be observed that a close relationship does exist between changes in pH and volatile acid content.

The activity of the glucose fermenting micro-organisms drops in three to four days to less than a quarter of its original value, e.g. half-time increases from five to ten hours to 24 to 60 hours. The lotaustralin hydrolysing micro-organisms seem to be much more sensitive to starvation, the decrease in activity being on the average considerably greater than for glucose and in one instance being sixty-fold. Nevertheless a close parallelism exists in the way the decrease occurs with each substrate. It should be borne in mind that the actual metabolic activity

of the micro-organisms at the pH of the rumen would be many times lower than those measured *in vitro*. After three to four days of starvation, the pH is increased from a normal value of 6.0 - 7.0 to the vicinity of 8.0. At this pH the activity of the lotaustralin-hydrolysing micro-organisms is reduced by a factor of five over those measured *in vitro* and recorded in this work. If the glucose and lotaustralin were introduced into the rumen itself the initial rate of breakdown would be very slow.

When feeding is recommenced after three to four days of starvation one difficulty has been to persuade the sheep to eat rapidly. The longer the starvation period the less rapidly did the sheep eat.

A close parallelism is observed in the return to normal ruminal conditions. When pH, volatile acid content, activity towards glucose and lotaustralin are plotted a very good agreement is obtained. When half-times are plotted (as in Fig. 2) the recovery of ruminal activity after starving appears to proceed rapidly right from the commencement of feeding. This can be misleading. If the true activity, as measured by the inverse of the half-time is plotted, the recovery proceeds slowly at first, then gathers momentum and later slows down again until maximum activity is reached. This is in fact the result one would expect if the recovery of ruminal activity is due to multiplication of the micro-organisms and a return to normal pH.

The general tendency has been for the activities to reach peak values which are coincident in time with peak values for volatile acids and minimum values for pH. This time is of the order 18 $\pm$ 6 hours. If by normal activity after starvation is meant the level applying before starvation commenced, the time would be slightly less, since there was a tendency for the activity reached in recovery to exceed that occurring before starvation. But in general the return to normal took at least 12 hours and at most 24 hours. As the volume of the ruminal contents had in most cases returned to the normal value of three to four litres by 12 - 24 hours, total volatile acid production and total microfloral counts as distinct from concentration, would also be normal. No significant differences due to type of diet fed before or after starvation were observed. The lucerne hay used was of high quality so that insufficient variation in readily digestible material was available in the diets used. Iggo (11) found that volatile acid production from good lucerne hay was comparable to that from succulent feeds, but that when poor quality hay was fed to sheep there was a decrease in volatile acid production. Any differences in rates of recovery would have to be of considerable magnitude to be detected by such crude methods as used in the present work. The time of recovery quoted is not in agreement with the observation of Quin (5) who considered that ten days were required for recovery after a three day starvation period. The agreement between pH, volatile acid content and microfloral activity towards glucose and lotaustralin, together with observations on the feeding habits of the sheep in this work, all confirm that recovery is complete within one day.

It has been shown in the present work that the rate of release of free hydrocyanic acid from lotaustralin by ruminal micro-organisms is greatly reduced by starvation. But when food in the form of dried grass is given there is, *in vitro*, at first inhibition, and later a rapid acceleration in rate of HCN production. There is also *in vivo* evidence

in support of this (2). In the normal animal, grazing steadily, there would be a fairly uniform rate of intake and release of any toxic substance. But with hungry animals, the rumen may become quickly filled, so building up a large concentration of the toxic substance. The release and absorption of this toxic substance, instead of being fairly uniform, may begin very slowly and then increase rapidly. Where the release of the toxic substance, either from the plant tissue itself or from an inert compound (such as a cyanogenetic glucoside) is facilitated by the ruminal microflora, it is probable that the factors mentioned above contribute to the danger of hungry stock gaining access to potentially toxic plants.

It is well known that losses in sheep and cattle can occur when animals, starved for a few days or even transferred from poor grazing, gain access to lush feed. The rapid rate at which microfloral activity builds up and the fact that the activity often reaches peak values exceeding those obtaining before the starvation began, suggests that this excessive fermentation might be a contributing factor. When the feed given is fibrous, one would expect, and one finds in practice, that the danger is not great, but when the food is succulent and contains much readily fermentable material the danger may be considerable. It is evident, therefore, whether the grazing contains toxic plants or not, that it would be wise to restrict the feeding of hungry ruminants to permit the rumen to adapt itself gradually to the changed rate of food intake.

ACKNOWLEDGMENTS

The author wishes to thank the University of New Zealand and the Department of Scientific and Industrial Research for financial assistance, and J. A. Cross for assistance with chemical analyses.

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METHODS FOR DETERMINING THE VIABILITY OF VARIOUS SEEDS BY TETRAZOLIUM STAINING

I. CHEWING'S FESCUE

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(Received for publication, 17th March, 1949)

Summary

This publication is the first of a series covering an investigation into the possibility of adapting Lakon's topographical method of determining viability for use with small seeds of importance in the New Zealand trade.

A technique is described which enables the germinating capacity of Chewing's fescue seed to be estimated within 48 hours.

The method includes pre-soaking the seed for 16 hours at 30°C., piercing the pericarp, and steeping the seed for 16 hours in 1 per cent solution of 2:3:5-triphenyltetrazolium bromide at 30°C.

The method of examining embryos and classifying them according to the distribution of the stain is described.

The percentage of seeds estimated to be viable by the staining method showed a high correlation with the percentage of viable seeds determined by germination test, the correlation coefficient being .985.

The standard error of the estimate of germination percentage from staining percentage was $\pm$ 5 per cent.

Introduction

The method of determining the viability of seeds by germination test generally gives satisfactory and conclusive results; but with many kinds of seeds an excessively long time is required to ascertain the full germinating capacity, and the need for a more rapid method has long been felt. The time required to complete a germination test may extend to several months in the case of those tree seeds which are characterized by intense dormancy, and which do not respond quickly to after-ripening treatments. It is not surprising therefore that much of the early work, aimed at developing a rapid bio-chemical method of determining viability, was carried out with seeds employed in forestry. This work has been adequately reviewed by Baldwin (1) and reference is made only to the staining methods developed by Hasegawa and Eidmann which were based upon the reduction of telluride and selenite salts in the cells of living plant embryos.

Using the selenium stain Lakon (2) developed what he termed the topographical method of determining the viability of seeds, and showed that a quick and reliable estimate of germinating capacity could be obtained by its use with the common cereals. In the use of vital stains for determining viability a difficulty arises out of the fact that in some seeds the embryo is only partially coloured. It is necessary in such cases to have some criterion of viability and it is in this respect that Lakon's method is of special interest. The criteria of viability employed by Lakon were based upon two assumptions. In the first place, it was assumed that all the embryo tissues which were shown by the staining reaction to be the seat of an active enzyme system were alive and capable of fulfilling their normal functions, whereas the tissues which did not react to the stain were dead or at least moribund and incapable of growth. In the

second place, it was assumed that a seed was viable so long as all parts of the embryo essential for the development of a plant and not capable of being regenerated remained alive, and that when some vital part of the embryo died, only an abnormal seedling capable of continued growth could be formed. From a study of the anatomy of the embryo and of the development of the seedling Lakon deduced for each kind of seed handled what parts of the embryo should be regarded as vital.

In 1941 Kuhn and Jerchel (3) drew attention to the properties of some tetrazolium salts and suggested that they could be employed in physiological studies as reduction indicators. The salts which were colourless in aqueous solution were reduced by some phytochemical processes to stable red coloured formozans. This reduction was observed to take place in the germinating seeds of *Lepidium sativum* and it was suggested that tetrazolium salts might therefore provide a substitute for the highly poisonous selenium salts, in the biochemical tests for seed viability.

Acting upon this suggestion, Lakon (4) experimented with eight different tetrazolium compounds. He found 2:3:5-triphenyltetrazolium chloride especially suitable for locating the tissue in which the reducing enzymes were active, because the triphenylformozan produced by the reduction of the salt did not readily diffuse from the cells where it was formed. As a result of these studies, Lakon discarded the selenium compound and adopted the tetrazolium salt for his topographical method for determining the viability of seed.

The procedure recommended by Lakon (5) for determining the viability of maize involved the following steps:—

The grain was soaked overnight and then bisected longitudinally through the embryo, and half of each grain steeped for at least three hours in a 1 per cent. solution of triphenyltetrazolium chloride at room temperature. The seeds were regarded as viable if the plumule, embryo axis and central portion of the scutellum reacted to the stain. The results were obtained within 24 hours and showed a satisfactory agreement with germination counts.

The reliability of the tetrazolium staining method for estimating the germinating capacity of cereals has been confirmed by Porter *et al.* (6) and Cotterell (7). Goodsell (8) found the method unreliable when applied to immature maize which had been exposed to an injurious degree of frost unless the grain was dried preparatory to staining. Porter *et al.* (6) found that the tetrazolium stain gave promising results with *Paspalum notatum* only when the method of application was considerably modified. They recommended that the seed should be pre-soaked and then held for three to four days under moist conditions with night and day temperatures of 20°C. and 35°C. respectively preparatory to staining.

Because it involves the bisection of large numbers of embryos, Lakon's procedure is better adapted for use with large seeds such as the common cereals. With small seeds it tends to become so slow and difficult as to be impracticable. Cottrell (9) concluded that the method could not be applied to small seeds.

The present studies were undertaken to ascertain whether the topographical method could be adapted for practical use with the small seeds of several species which are of special interest to the seed trade in New Zealand. The first species to be studied was Chewing's fescue.

The seed of Chewing's fescue *(Festuca rubra var. commutata Gaud.)* is less retentive of viability under ordinary conditions of storage than most other seeds commonly employed in agriculture, and for this reason those who handle the seed require to test its germinating capacity frequently.

Chewing's fescue seed, although not exhibiting persistent dormancy, often fails to germinate promptly and evenly. Kearns and Toole (10) found that under optimum conditions a period of approximately 21 days was required to ensure the germination of all viable seeds.

A more rapid means of measuring the germinating capacity of Chewing's fescue seed would be useful in many circumstances. Particularly when lines of seed of exceptionally high moisture content are being conditioned for export in driers employing heated air, there are occasions when it is desirable to determine as soon as possible whether any considerable loss of viability has been incurred.

EXPERIMENTAL METHODS

Forty-two samples representing different commercial lines of Chewing's fescue seed were employed in the present studies. No sample was more than 18 months old and almost all were of high germinating capacity. It was considered desirable to have a wider range of germinating capacity, and this was achieved by repeating treatments used in a previous study (11) of the influence of heat and moisture on the rate of decline in germinating capacity of Chewing's fescue seed. The samples in three lots were placed in atmospheres at different levels of relative humidity for 20 hours, and then all exposed to a temperature of 77°C. for 15 minutes.

Germination tests were carried out on 300 seeds counted at random from a portion of the sample freed of all impurities. Night and day temperatures in the germinating incubator were 16°C. and 30°C. respectively, the higher temperature being maintained for eight hours. The testing period was 21 days.

In Chewing's fescue seed, which has imbibed water to the point of saturation, the embryo is approximately 0.6 mm. long, and on account of their small size it was found difficult to bisect large numbers of embryos accurately. An alternative method of applying the tetrazolium stain to that recommended by Lakon (5) was therefore sought. The method adopted involved the following steps:—

(*a*) From a portion of the sample from which all impurities had been removed, approximately 300 seeds were taken at random and soaked by floating on the surface of tap water for 16 to 20 hours at 30°C.

(*b*) The water was drained off and the pericarp of each seed was then punctured with a dissecting needle, care being taken to avoid injury to the embryo.

(*c*) The seeds were then disinfected by steeping them for 10 minutes in a solution of calcium hypochlorite.

(*d*) After washing in distilled water the seeds were transferred to a small glass dish and covered with 1 per cent. solution of 2:3:5-triphenyltetrazolium bromide, and placed in a darkened incubator at 30°C. for 16 to 24 hours.

(*e*) The seeds were then washed in tap water, transferred to a petri dish, and examined in water with a binocular dissecting microscope at magnifications of 10 x to 30 x.

(*f*) Whenever necessary the embryo was dissected out. This was easily done by breaking the lemma at its base where it joins the callus and then pressing down upon the lemma with a dissecting needle just above the top of the scutellum.

For the purpose of studying the distribution of stained and unstained tissue, some of the excised embryos were bisected, longitudinally as shown in Figure I. However, it should be noted that the method employed for distinguishing viable and non-viable seeds was based upon the distribution of staining visible at the surface of the embryo and that embryos were not bisected for the purpose of classification.

Most of the seeds fell distinctly into two groups, the first having the visible surface of the embryos stained all over, and the second having embryos without visible staining. Seeds in the first group were classed as viable, those in the second as non-viable. In both cases the condition of the embryo could generally be seen satisfactorily through the translucent wet lemma.

In every sample there were in addition some seeds in which only parts of the embryo were stained. These embryos were always dissected out for closer inspection. They were classed as viable if the plumule, the embryo axis down to the epiblast and a portion of the scutellum amounting to at least a third of the visible area and extending inward to the plumule were all stained, *e.g.*, Fig. 1, A to C. Other cases of partial staining were classed as non-viable, *e.g.*, Fig. 1, D to L.

Results.

In Table I the results of comparative tests on the 68 samples of Chewing's fescue seed are shown. The first 42 samples represent different commercial lines of seed. Samples 43 to 68 were from lots of seed that had been treated by heat to bring about a rapid reduction in germinating capacity. The same data are represented in the scatter diagram, Fig. 2.

Discussion.

The samples tested have been dealt with in two groups, the untreated samples and those which had been subjected to heat treatment. It was found that these groups differed with regard to the distribution of staining with the embryo and in the regression of germination percentage on staining percentage.

Lakon (2) observed that in the embryos of wheat, barley, and oats which had begun to decline in vitality there was a progressive extension of necrosis from the tip of the main root primordium upwards to the epiblast, and commencing later a development of necrosis in the tip of the plumule spreading downward and eventually involving the whole embryo. He also found that a similar development of necrosis could be induced artificially by immersing soaked grain for several hours in water at 50°C.

Observations on the untreated samples of Chewing's fescue seed indicated that the development of necrosis within the embryo normally follows a similar course. However, when Chewing's fescue seed in the air-dried condition was exposed for 15 minutes to a temperature of 77°C., the position with regard to the distribution of necrotic tissue was quite different. There was no evidence of a consistent spread of necrosis from the tip of the root primordium upwards. Areas of living and necrotic tissue were variously distributed throughout the embryo. An indication of the variety of stain distribution is given by the selection of embryos

illustrated in Fig. 1. There the distribution of staining in both the surface aspect and the median longitudinal section is represented. In Fig. 1B the lateral portions of the scutellum have lost vitality first. In Fig. 1G the entire scutellum is necrotic, while the plumule, axis, and root primordium still remain alive. Fig. 1F is similar, but necrosis has extended to the embryo axis. In these cases it is apparent that the onset of necrosis does not follow the normal course.

The distribution of necrotic tissue in heat-treated Chewing's fescue seed was similar to that observed by Lakon (4) in maize samples of low vitality. Lakon regarded the difference between the spread of necrosis which he observed in maize and that observed in the other cereals studied as being characteristic of the species, but since both types of distribution have been observed in Chewing's fescue seed it appears that the order in which the different embryo parts lose their vitality depends in some degree upon the nature of the environmental factors influencing loss of vitality.

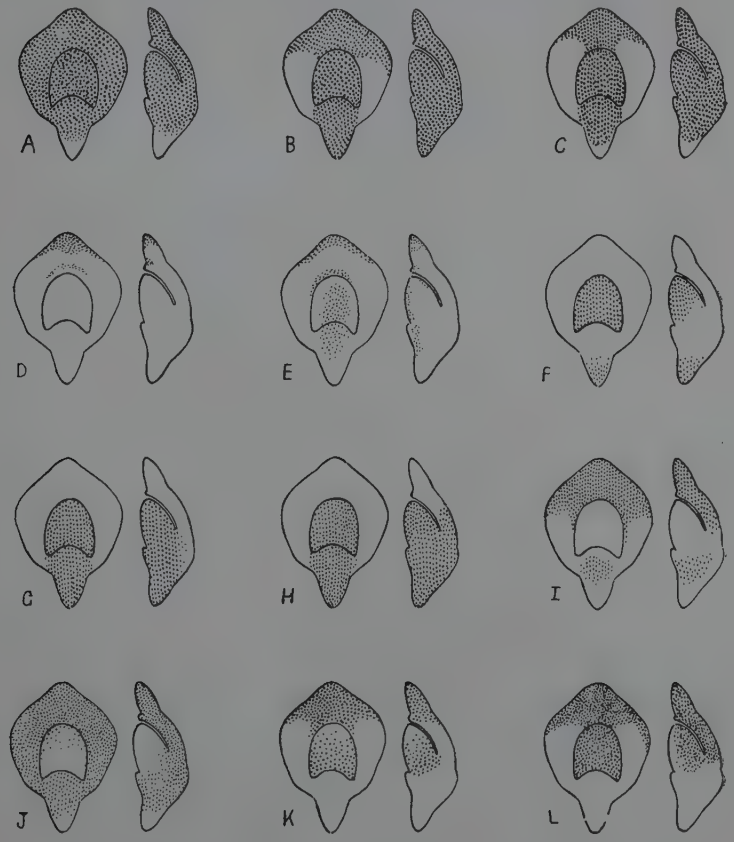

FIG. 1.—Embryos of Chewing's fescue seed, showing partial staining, with surface aspect and median longitudinal section represented. A to C classified as viable; D to L classified as non-viable.

The regression of germination percentage on staining percentage shows that with the heat treated samples of higher germinating capacity there was a tendency for a small proportion of seeds incapable of producing normal seedlings to be classed as viable by the staining method. It appears therefore that the criteria of viability employed in the staining method were not entirely satisfactory when applied to the heat-treated samples and call for reconsideration, particularly with regard to the propriety of classifying as viable the partially stained embryos represented in Fig. 1 by examples A to C.

In formulating the criteria of viability, it was assumed that the portions of the Chewing's fescue embryo which are vital for the production of a seedling capable of continued growth were the plumule, the embryo axis, and a central portion of the scutellum maintaining continuity between the embryo axis and a living portion of the epithelial layer. It was also assumed that when these parts react to the stain the seed is capable of normal germination.

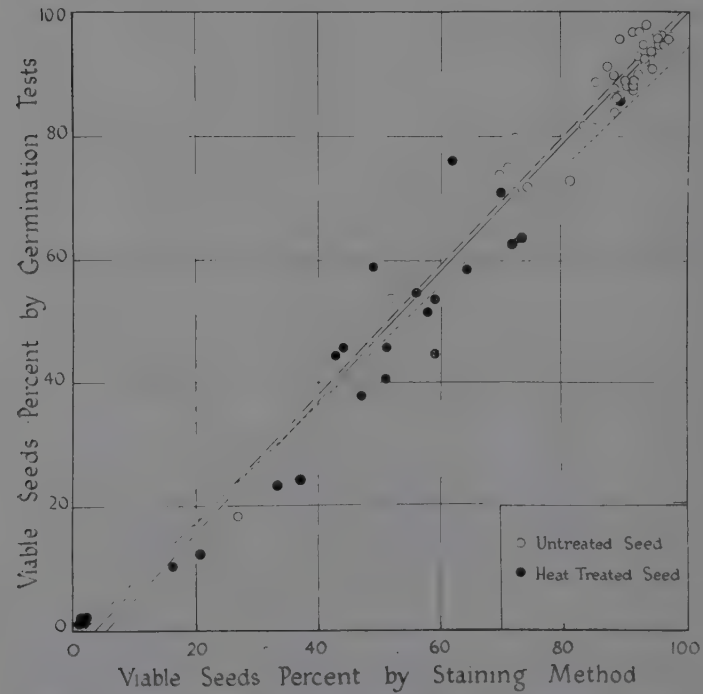

FIG. 2.—Scatter diagram showing the relation between germinating capacity determined by germination test and the estimation by the tetrazolium staining method.

Total samples - - - - - - $y = -4.642 + 1.047x$ Correlation .985
Untreated samples - - - - - $y = -4.086 + 1.046x$ Correlation .966
Heated samples - - - - - $y = -3.093 + .997x$ Correlation .967

The parts of the embryo not considered essential were the coleorhiza, the root primordium and the peripheral portion of the scutellum. Seedlings from which the main root had been removed produced one or two adventitious roots from the scutellar node and therefore it was considered that the necrosis of the root primordium did not in itself render a seed non-viable.

Previous workers, including Lakon (4), Cottrell (9), and Goodsell (8) had found that in cereals normal germination took place when portion of the scutellum was necrotic. However, Lakon (4) found that necrosis of the scutellum had an inhibiting effect upon the development of the embryo beyond what was attributable to the failure of part of that organ to function, and this aspect calls for further study.

Another possible source of error lies in the fact that condition of the embryo was judged from the distribution of staining visible at the surface. A preliminary study in which the distribution of staining visible at the surface was compared with the staining visible in a median longitudinal section indicated that when as much as a third of the outer surface of the scutellum was stained the more vital deep-seated tissue connecting the embryo axis and the epithelial layer was also stained. The bisection of approximately 200 embryos failed to produce any instance which did not accord with the rule. On the other hand, there were two cases like that illustrated in Fig. 1H, where the whole of the visible surface of the scutellum was unstained but the deep-seated central portion was stained.

The method of estimating the germinating capacity used in the present study was more satisfactory with the untreated seed than with the heat-treated samples that contained a large proportion of partially stained embryos in which the distribution of necrotic tissue was irregular. A further investigation of the effects of necrosis of the scutellum in grass embryos is called for, but this could be carried out best with a species having a larger embryo than that of Chewing's fescue.

From the results of tests on 64 samples, it is calculated that the standard error of estimation of germinating capacity from staining percentage is $\pm$ 5 per cent. The tetrazolium staining method of estimating germinating capacity should be useful as a rapid means of detecting any serious loss of viability in Chewing's fescue seed resulting from drying or other treatment. If the seed were tested before and after such treatment a difference between tests could be regarded as significant at the 19:1 level if it exceeded 14 per cent. A smaller loss of germinating capacity could be detected by using the conventional germination test. When these tests are based upon 300 seeds and the germinating capacity is approximately at the 50 per cent level it can be expected that differences exceeding 8.5 per cent will be significant. The reliability of estimates of germinating capacity can be increased by the replication of tests. With the tetrazolium staining method a system of sequential sampling can be conveniently employed to ensure that the number of seeds examined in each case is sufficient to give significant results.

Acknowledgments

The assistance of Messrs May and Baker Ltd. and Mr R. W. Bailey in supplying samples of tetrazolium salts is acknowledged.

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TABLE I. RESULTS OF COMPARATIVE TESTS FOR GERMINATING CAPACITY OF 64 SAMPLES OF CHEWING'S FESCUE SEED BY THE TETRAZOLIUM STAINING METHOD, AND THE STANDARD GERMINATION TECHNIQUE.

Sample.	Germinating Capacity.		Sample.	Germinating Capacity.	
	Estimated by tetrazolium staining.	Determined by germination test.		Estimated by tetrazolium staining.	Determined by germination test.
1	92	93	35	95	96
2	90	88	36	95	96
3	85	89	37	93	92
4	89	96	38	88	84
5	93	95	39	95	95
6	87	91	40	91	89
7	92	97	41	95	97
8	83	82	42	90	89
9	88	90	43	74	72
10	94	96	44	59	54
11	85	83	45	71	75
12	27	18	46	72	63
13	72	71	47	64	59
14	72	80	48	48	59
15	52	54	49	56	55
16	89	87	50	89	86
17	95	93	51	62	76
18	97	96	52	43	45
19	93	98	53	51	46
20	91	97	54	59	45
21	94	91	55	51	41
22	92	90	56	70	71
23	97	96	57	37	24
24	91	87	58	58	52
25	70	74	59	44	46
26	93	93	60	72	63
27	95	88	61	47	38
28	89	87	62	33	23
29	87	86	63	21	12
30	87	88	64	16	10
31	81	73	65	2	1
32	96	95	66	1	0
33	94	94	67	0	0
34	91	88	68	0	0

CONTROL OF FERMENTATION IN HONEY BY INDIRECT HEATING AND DRYING

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(*Received for publication, 4th August, 1948*)

Summary

The main cause of fermentation in extracted honey is shown to be excessive moisture present in honey combs apparently ripe for extraction. Experimental work is described which led to the construction of a drying-plant capable of removing this moisture and so controlling fermentation. Indirect heating and drying is used in the plant.

Introduction

Loss of flavour and condition in honey is caused almost entirely by fermentation brought about by the growth of sugar-tolerant yeasts. Such yeasts occur in the nectar of flowers visited by bees (1), in the soil surrounding apiaries (2), and on the bodies of bees (3). They are transported by bees from these places to the combs in the hives. It is apparent that, for practical purposes, all extracted honey may be considered to contain yeasts, the entry of which cannot feasibly be prevented. Lochhead and Heron (1) isolated honey-fermenting yeasts from honey tanks, containers, and the air of the extracting room, and Marvin (4) considers that they are present in almost all honeys. Usually yeast growth is inhibited by the high sugar concentration of honey and the yeasts die out.

When the moisture content of honey increases above certain limits, however, the concentration of sugars is so reduced that yeasts can multiply and fermentation is more likely. This fact, pointed out by Dyce (5) and Lochhead and MacMaster (6) has been repeatedly confirmed in other countries. Observations made by the authors of this paper also confirm the association of high moisture content with fermentation in New Zealand.

Since practically all New Zealand honey is marketed in a granulated condition, and as it is well known that granulated honey is more likely to ferment than liquid honey, the presence of excess moisture is of particular importance. The tendency for granulation to favour fermentation is explained by Dyce (5) as being due to crystallization of the dextrose in honey as dextrose hydrate which contains a much smaller percentage of water than is usually present in the honey. This results in an increased water content for the remaining liquid phase which favours fermentation.

Honey-producing countries provide a maximum permissible moisture content in grading honey for export, mainly to reduce the risk of fermentation.

Honey that has passed through a stage of fermentation will not respond to treatment. If the yeasts are killed by heat or drying the sour flavour still remains. The honey can be improved only by blending it with sufficient normal honey to mask the flavour.

Fermentation in extracted honey may be prevented by two methods.

The first, consisting of heating the honey sufficiently to destroy yeasts, is much practised in the U.S.A., where almost all honey is sold as liquid, pasteurized honey. There is a strong prejudice against granulated honey in the U.S.A., where an additional advantage of the treatment is that it produces a liquid product. Apart from drawbacks due to the somewhat elaborate equipment required for pasteurizing, and the care necessary to prevent overheating of the honey, liquid honey is not popular in New Zealand.

For these reasons the authors developed a second method which depended on treating honey so that it would not support yeast growth. This was finally achieved by designing a plant that would remove excess moisture from the honey by indirect heating and drying, first in a comb drying-room, and then, after extraction, in a tank drying-room.

Temperatures, too, have an effect on yeast growth, but in New Zealand no attempt is made in the commercial handling of honey to keep it at temperatures unfavourable to multiplication of yeasts.

The first step necessary in devising an alternative to pasteurization for prevention of fermentation in extracted honey was to determine the origin of the excess moisture which was the main cause of the trouble.

Experimental work which resulted in solving the problem is outlined in this paper.

EXPERIMENTAL METHODS

Preliminary observations were made to determine whether high moisture content in honeys was associated with high yeast count. In carrying out yeast counts the following method was employed :—

The honey sample was first thoroughly mixed, using a sterile stainless steel spatula. Then 10 g. was weighed into the bottom of a straight-sided bottle and distilled water run in until the level reached a mark denoting a total volume of 20 ml., *i.e.* 0·5 g. honey per ml. The sample was then thoroughly mixed and serial dilutions prepared up to 1/1,000, or further if necessary, using 50 per cent by volume honey-solution (pH 6·8 to 7·0) as a diluent. The dilutions were plated out in duplicate, using 50 per cent by volume honey-agar (7). The yeast colonies which appeared in appropriate plates after incubation at 26°C. were then counted, using a hand lens or dissecting microscope. The usual bacteriological precautions against contamination were observed throughout.

The figures shown in Table I were obtained by the use of this method. They are representative of many samples of extracted honeys and confirm the work done overseas.

The generally accepted view has been that fermentation of extracted honey through high moisture content is due to the absorption of moisture from the atmosphere by the honey during extraction and while in the settling tanks. Most of the absorption was thought to occur in the top layer of the honey in the tanks. This explanation was adopted mainly because it was known that the top layer of honey in a settling tank usually had a lower gravity than the bottom one. See Table II.

Table I. Relationship between Moisture Content and Fermentation

Percentage of Moisture.	Specific Gravity.	Yeasts per Gram Honey Immediately after Extraction.	Fermenting Honeys.		Non-fermenting Honeys.	
			Period in Months from date of extraction to commencement of fermentation.	Yeast Counts when fermentation commenced.	Period in Months from date of extraction to time yeast counts made.	Yeast Counts.
15.6	1.431	800	—	—	10	34
16.8	1.422	705	—	—	17	0
17.0	1.421	1,514	—	—	17	0
17.2	1.420	868	—	—	16	0
17.2	1.420	1,324	—	—	17	4
17.3	1.419	1,059	—	—	17	4
17.4	1.418	7,060	6	17,330	—	—
17.8	1.416	161	9	98,000	—	—
17.8	1.416	164	9	651,000	—	—
17.9	1.415	10,350	9	159,500	—	—
18.0	1.414	12,880	6	1,929,000	—	—
18.0	1.414	2,740	11	9,180,000	—	—
18.2	1.413	5,300	9	89,000	—	—
18.4	1.412	3,300	6	71,400	—	—

Table II. Specific Gravity Determinations on Top and Bottom Layers of Honey in Settling Tanks

Location of Extracting Plant.	Percentage Moisture in top layer honey in settling tank.	Percentage Moisture in bottom layer honey in settling tank.	Difference.
North Auckland	17.8	17.4	.4
,, ,,	17.2	16.8	.4
South Auckland	17.2	15.4	1.8
,, ,,	16.4	16.0	.4

The theory of absorption of water at surfaces was checked by exposing various honeys on waxed boards to determine the rate of absorption of moisture. Results showed that penetration of moisture under the most advantageous conditions was limited to a thin surface film which would be removed by the usual skimming of settling tanks.

It was apparent that absorption of moisture by tank honey could not account for the excess moisture often met with after extraction.

Although it has been recognized in America that fully capped and apparently ripe combs removed for extraction may contain honey with excess moisture (8), the problem has not been studied there in detail. The occurrence of similar conditions on a large scale had not previously been suspected in New Zealand, but was thought by the authors to account for the high moisture content of the top layer of tank-honey. This would result through separation of such comb-honey after extraction in the ripening tanks, the portion with a high moisture content and consequently a lower specific gravity rising to the surface.

In testing the theory it was first necessary to show that excess moisture did in fact occur in combs apparently ripe for extraction.

Presence of excess moisture in fully capped combs

The Chataway honey hydrometer will register the moisture content of one pound of honey cut from the comb and strained, but the result

is an average, covering portions of honey that may be normal and those that may contain an unusually high percentage of moisture. It should be noted, moreover, that the high temperature, 122°F., to which honey is heated for the test, prevents the formation of layers, and gives a homogeneous sample. It was thought that small areas of comb might often contain excess moisture which the Chataway hydrometer, depending on a composite sample, would not detect. A refractometer (9) was not available for these preliminary determinations and so a flotation test, now described, was designed to test the specific gravity of small volumes of honey (approximately 28 ml.).

Flotation test :

Ordinary honey samples were first tested by the honey hydrometer to give a range of standards of known moisture content. Then small sections were removed from the comb under test and placed in jars with liners. Heat was applied and the wax brought to the surface and removed, leaving a sample of approximately 56 ml. of honey, sufficient for two tests. A small volume of one of the standard honeys was run into a test-tube, and a small sample of the comb-honey under test run on to its surface. Even if both samples were slightly stirred, separation occurred, the lighter bodied honey rising to the surface. Experimentally, separation was shown by using honeys of different colour. If this was impracticable, a few crystals of a dye such as crystal violet were used to dye the sample whose moisture content was being determined. Honeys of varying moisture content will not separate once they have been heated to a temperature above 160°F. In practice it was found that a temperature of approximately 95°F. facilitated separation.

The test was repeated, using different standards, until representative samples from the whole comb had been covered. The test was also used to compare honeys from different areas of the same comb against each other. Results commonly showed marked variation in moisture content for different areas of the same comb, the moisture in many cases being excessive.

The test is accurate only within certain limits but it was capable of showing that considerable variation in moisture content usually occurs between different areas of honey on the same comb, and that excess moisture is often present.

Fermentation and high yeast count in fully capped combs

High moisture content of honey and fermentation are related, and consequently it would be expected that, as much honey apparently ripe for extraction contains excess moisture, it would also frequently show signs of fermentation.

Yeast-counts on nine combs from individual hives in the Laboratory apiary, Wallaceville, resulted as follows : six combs were fermenting with yeast-counts ranging from 100,000 plus to 100,000,000 yeasts per gram, while, for the remaining three combs, yeast-counts were 632, 16,850, and 26,350, yeasts per gram.

Amongst other examples of high yeast-count six samples of newly stored fully capped comb-honey from individual hives of one producer gave yeast-counts ranging from 19,400 to 77,200 yeasts per gram.

The following results were obtained from an examination of samples of fully capped comb honey collected near Auckland on 2/5/47 from six hives. See Table III.

Table III. High Moisture Content of Fully Capped Comb Honey associated with High Yeast Count

Source of Sample.	Percentage Moisture by Chataway Hydrometer.	Yeasts per Gram.
Bottom super	17.8	2,183
Bottom super	18.2	3,346
Second super	17.7	9,580
Bottom super	21.4	1,256
Second super	20.2	12,830
Bottom super	20.6	30,830

Active fermentation was in progress in many cells of the combs from which these samples were collected, bubbles of gas being evident. In obtaining sufficient honey (1 lb.) for carrying out the Chataway test it was necessary to include non-fermenting honey which would depress the yeast-count.

Later in the investigation, a refractometer was available and its use enabled small samples (20-40 g.) to be removed from combs for both specific gravity determinations and yeast-counts In collecting such samples, for convenience of reference, each comb was considered divided into three portions of equal area, the middle section being denoted by the letter 'B' and the two end areas by 'A' and 'C'. Samples were removed from only one side of the comb. Table IV gives results of an examination of combs removed from different hives in January and February 1948. These combs were fully capped and apparently ripe for extraction, being situated in supers above the brood chamber. The table illustrates marked variation in moisture content and yeast-count between different parts of the same comb.

Table IV. Variation in Moisture Content and Yeast Count between Different Areas of Fully Capped Combs

Comb No.	Area of Comb Sampled.	Moisture Content by Refractometer (Average of ten readings). (Per cent.)	Yeasts per Gram.
1	A	19.2	1,980
	B	17.2	147
	C	18.7	14,700
2	A	22.2	355,000
	C	21.7	7,350
3	A	18.3	0
	C	17.7	156
4	A	19.1	1,923
	C	20.5	26,700
5	A	16.3	18
	C	17.9	932
6	A	16.0	4
	C	15.7	0

Sufficient evidence has been collected to indicate that in many instances honey may be of low gravity while in capped combs on the hive. Yeast-counts on comb honey removed from hives are often very high, even when the honey is not actively fermenting. While such honey normally may constitute only a small proportion of the total passing through an extracting plant, its presence is of great importance for the following reasons:—

(i) If fermentation is occurring in the combs they will contribute a large inoculum of yeasts to the bulk honey being extracted. This will tend to hasten fermentation in the extracted honey if conditions become suitable for yeast multiplication (6).

(ii) Sufficient fermented honey may enter the bulk honey during extraction to give it a sour taste even if conditions are such that the extracted honey will not support the growth of yeasts.

(iii) The most important effect of such low gravity comb honey is often to depress the specific gravity of extracted honey to an extent liable to favour fermentation.

Stephen (10) described a method of removing moisture from comb honey in which supers of combs were placed on top of a horizontal steam radiator, over which air was forced by a multivane fan. The rate at which the hot air flowed was 760 ft. per minute. Forced draft was considered necessary to transfer the heat to the combs and carry away the moisture. It was found possible to remove approximately 1.35 per cent of moisture from combs in twelve hours. Heavy combs, fully capped, lost little moisture. The plant had not passed the experimental stage.

It appeared feasible to the authors to control fermentation in extracted honey by drying the combs before extraction in a comb drying-room, and housing the settling tanks in a tank drying-room where further removal of moisture from the extracted honey would occur. Because moisture would be removed by circulating air of low relative humidity, all heat applied to the honey would be indirect, and colour and aroma would not suffer. It was considered that the specific gravity of extracted honey could be raised sufficiently to prevent the growth of yeasts.

Accordingly, a miniature plant for drying honey was constructed and experimented with on these lines. A drying-room with a thermostat set at 90°F. so that a fan and heating elements cut off together, was used to reproduce large scale conditions. The internal dimensions of the insulated drying-chamber were 14 in. × 14 in. × 14 in. The thermostat was a mercury regulator with a battery-operated relay. The fan, operated by a hundredth of a H.P. electric motor running at 1,300 R.P.M., gave a vigorous circulation of air, warmed by a small heating element. By this arrangement relative humidity was maintained at approximately 35 per cent inside the drying-chamber.

The first series of experiments were arranged to study the effect of drying on honey combs placed in a comb drying-room before extraction.

Drying of honey in comb drying-room

Uncapped combs:

The cells in these combs were filled with a honey of known moisture content and when the test was completed the honey was strained from the wax and the moisture content re-determined. The honey used was from Southland Birch, Canterbury Clover, and mixed sources.

Table V. Drying of Uncapped Comb Honey in Miniature Comb Drying-room

Comb No.	Time of exposure. (hr.)	Moisture (Readings by Chataway Hydrometer).		Moisture. lost. (per cent.)
		Before drying. (per cent.)	After drying. (per cent.)	
1	23½	18.4	17.2	1.2
2	41	18.4	16.4	2.0

Capped combs :

Fully capped combs of honey were cut in half, the honey from one half being tested for moisture, and the other half being exposed, as before, to drying before testing. The honey was from mixed Hutt Valley sources and contained a percentage of Manuka honey.

Table VI. Drying of Fully Capped Comb Honey in Miniature Comb Drying-room

Comb No.	Time of exposure. (hr.)	Moisture (Readings by Chataway Hydrometer).		Moisture lost. (per cent.)
		Before drying. (per cent)	After drying. (per cent.)	
3	24	20	18.4	1.6
4	24	18.2	17.4	0.8
5	24	16.0	15.4	0.6
6	43½	18.4	15.4	3.0
7	41	17.2	16.6	0.6

Removal of excess moisture from honey combs before extraction is of primary importance, as even if yeasts are present in combs in large numbers they will not cause fermentation in the extracted honey if its specific gravity is increased sufficiently by drying. This is exemplified by the greatly reduced yeast count in the half of comb No. 5 subjected to drying. See Table VII.

Table VII. Effect of Drying on Yeast Count of Comb Honey

Sample.	Yeasts per gram. 13/9/46	Yeasts per gram. 1/10/46	Yeasts per gram. 6/11/46
Composite sample, composed of honey removed from six parts of half of comb No. 5 not put in dryer.	10,000,000 plus.	2,972,000	3,000,000
A similar sample from half of comb No. 5 dried out for 24 hr. in comb drying-room.	10,000,000 plus.	44,200	1,678

Capped combs responded well to conditions easily attainable in an efficient comb drying-room. Honey with only 16 per cent moisture was reduced to 15.4 per cent within twenty-four hours. Honey with 20 per cent. moisture was reduced to 18.4 per cent. Uncapped honey also dried out well during the same period.

Drying action is greater in a given time in a comb drying-room than in a tank drying-room, as a much greater area of honey is exposed.

The holding of combs in the warmth of a comb drying-room would facilitate extraction, particularly in the case of honeys which, owing to high viscosity, do not extract easily.

Drying of honey in a tank drying-room

Honey passing from a comb drying-room *via* the extractor into the tanks in a tank drying-room will immediately commence to form layers. This process, which is accelerated as the temperature builds up, is illustrated in Fig. 1 in which the honey tanks are represented by glass beakers of similar proportions, holding approximately one pound of honey. A high gravity honey (15 per cent. moisture content) was poured into the beakers, followed by a low gravity honey (18.4 per cent. moisture content). A few crystals of a dye were mixed into the honey of high specific gravity for sharp differentiation when formation of layers occurred. Honeys passing into tanks in a tank drying-room after treatment in a comb drying-room would not show such a marked difference in moisture content.

FIG. 1.—Reading from left to right 'A' shows a distinct separation of honeys in a miniature tank; 'B' shows the bottom layer of honey commencing to lift after prolonged exposure in a miniature tank drying-room; 'C' shows a later stage than 'B'.

It has been found that the use of a honey-pump for pumping honey from the extractor to the honey tanks does not materially affect separation.

As no direct heat is applied to honey during the operation of the plant, its effect on separation in the tank drying room is not considered.

When honey reaches a tank drying-room from a comb drying-room separation occurs, the remaining honey with excess moisture being concentrated in the top layer where the excess moisture is removed. If drying proceeds for a sufficient length of time, the top layer drops down in the tank, and its place is taken by another layer of honey which is dried in turn.

The same drying-chamber, run at 90°F., was used to simulate conditions operating in a tank drying-room in which light and heavy bodied honeys, run together into tanks, were exposed to drying.

Glass beakers, 7.5 cm. in diameter, and of 400 ml. capacity, were used as miniature tanks.

Two honeys, one of 18.2 per cent. moisture content (Clover source) and one of 16.0 per cent. moisture content (Manuka source) were used. The heavy bodied honey was poured in first, followed by the light bodied. In all cases the honeys soon separated, the light bodied honey forming a distinct top layer. Formation of layers was easily seen as one of the layers was light and the other dark in colour.

Beakers were weighed before the honey was poured.

In all cases the top layer was 3 cm. deep, and the beakers were exposed in the drying-chamber for 49½ hr. See Table VIII.

Table VIII. Drying of Honey in Miniature Tank Drying-room

Beaker.	Depth of Bottom Layer. (cm.)	Weight of Honey. (g.)	Loss in Weight. (g)	Loss in Weight. (per cent.)
A 1	5.3	479.9	1.95	0.40
A 2	5.4	490.7	2.1	0.42
A 3	5.4	482.9	1.9	0.39
A 4	5.5	486.1	2.1	0.43
A 5	5.4	485.5	2.1	0.43
A 6	5.0	474.9	2.0	0.42

A beaker of one honey (18 per cent. moisture content) left outside the drying-chamber remained unchanged for five days. The depth of honey in this beaker was 8·3 cm. During this period relative humidity and temperature measurements were made continuously by a thermo-hygrograph. The temperature varied between 74°F. and 58°F. and relative humidity between 80 per cent. and 60 per cent.

Figures obtained for loss in weight show good agreement.

The experiment was repeated, using one honey of uniform moisture content (18·4 per cent.). This allowed Chataway hydrometer readings to be taken at the end of the tests. The Chataway readings were taken, as such readings would be used in the field to check drying action. The results of the experiment are summarized in Table IX.

Table IX. Drying of Honey in Miniature Tank Drying-room

Beaker.	Tempera-ture.	Time of Exposure. (hr.)	Specific Gravity of Honey. (1.4115 at start of experiment.)	Loss of Moisture.	
				By Weighing. (per cent.)	By Chataway (Hydrometer.) per cent.
A	90°F.	42	1.418	0.9	
B	90°F.	42	1.416	0.58	0.6
C	90°F.	46	1.414	0.51	0.4
E	98.6°F.	49½	1.417	0.85	1.0
D	98.6°F.	143½	1.435	3.6	

Percentage loss in moisture by the Chataway hydrometer shows reasonably close agreement with figures obtained by weighing. Chataway hydrometer readings were not made for beakers A and D as these contained a large percentage of Manuka honey which affects the accuracy of the instrument.

It will be seen that tank honey responded well to conditions easily attainable in a tank drying-room, the loss in moisture content being slightly over 0.4 per cent. in 49½ hr. Using one honey of 18.4 per cent. moisture content higher values were obtained for moisture loss, due to decreased resistance to evaporation.

The results obtained from the miniature plant were so satisfactory that a large scale plant was put into operation. This plant which has functioned satisfactorily throughout several seasons in the North Auckland district has already been fully described by the present authors (11).

It is in two separate units, comb drying-rooms, and a tank drying-room. A temperature of approximately 95°F. is maintained in the comb drying-rooms, resulting in a relative humidity of from 40 to 45 per cent. In the tank drying-room corresponding figures are 97°F. and 40 per cent. These temperatures, slightly higher than in the experimental plant, were found more satisfactory for large scale plants.

The drying units in each room consist of three 1,000 watt elements fitted into a frame suspended diagonally from the ceiling across a corner of the room, and for air circulation a fan driven by a one quarter H.P. motor. The fan is placed in the corner of each room on top of an air chute so as to direct air drawn up the chute mainly through the space between the top element of the heater and the ceiling. This arrangement ensures efficient distribution of the heated air. A thermostat set at 95°F. is wired into the circuit so that fan and heater cut off together.

The drying unit was designed to work at its optimum in a comb drying-room of 10 ft. by 9 ft. with a 7 ft. stud, and a tank drying-room of 13 ft. 9 in. by 8 ft. with a 7 ft. stud. The capacity of each comb drying-room is 100 supers, stacked in criss-cross piles to assist air circulation. The capacity of the tank drying-room is 5,000 lb.

Exposure in the comb drying-room for from 24 to 48 hours is usually sufficient for fully capped combs. The same time suffices in the tank drying-room.

In New Zealand 17.2 per cent. moisture content is the permissible maximum for honey. The plant which was designed to remove moisture above this level functions successfully. It is considered that in its present stage of development the plant could, if necessary, remove up to 6 per cent. of moisture.

The plant is highly successful in removing excess moisture from extracted honey and so preventing fermentation after the honey is packaged. It will also arrest fermentation which may have already commenced in the comb-honey before extraction. The plant will not remove sour flavour in extracted honey caused by fermentation prior to extraction. While sour flavour is partially caused by seasonal factors beyond control, it is aggravated by the exposure of combs of honey to moist conditions, promoting yeast growth before extraction. Similar conditions will cause growth of yeasts in the residual honey in wet combs after extraction. Storage of these two types of comb in a comb drying-room prevents absorption of moisture, and, by its drying action, arrests active fermentation with resultant sour flavour.

ACKNOWLEDGMENTS

The authors wish to thank Mr. A. L. Bryant, Photographer, Animal Research Station, Wallaceville, who took the photographs shown in Fig. 1. Thanks are also due to Mr. R. S. Walsh, Apiary Instructor, Auckland, for collecting the samples referred to in Table III.

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THE MEASUREMENT OF FEED INTAKE BY GRAZING CATTLE AND SHEEP

I. A METHOD OF CALCULATING THE DIGESTIBILITY OF PASTURE BASED ON THE NITROGEN CONTENT OF FÆCES DERIVED FROM THE PASTURE

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(*Received for publication, 22nd December, 1948*)

Summary

It was observed from a series of digestibility trials, that the relationship between the weight of pasture organic matter consumed by sheep, and the total nitrogen excreted in the fæces, was almost constant.

Based on this observation, a method of calculating organic matter digestibility from the concentration of fæcal nitrogen has been developed.

Digestibility coefficients calculated by the proposed method are compared with those obtained from fifty-two digestibility trials.

INTRODUCTION

A KNOWLEDGE of the quantity of feed consumed by animals is necessary to the progress of many investigations into animal production problems. No serious obstacles in the measurement of this quantity are likely to arise when animals are fed weighed rations; but under conditions of free grazing, the determination of feed intake presents considerable difficulties. New Zealand is a country in which animal production is based almost entirely on grazing. Therefore, in studies which must of necessity take into consideration interactions within the system, soil—pasture—animal, it is essential that an accurate and practical technique for measuring intake of feed by the grazing animal should be developed.

The solution of innumerable problems is delayed by lack of a suitable method of making this measurement. The estimation of pasture yield has been attempted in this country by various methods which have been reviewed by Lynch (1947). Some of these methods utilize the grazing animal as measuring device, but as yet no entirely satisfactory means of measuring the animal's intake appears to have been employed, although the methods described by Sears (1944) have proved useful with sheep, and have been adapted by Wallace at Ruakura with cattle on a group basis. Much less laborious methods, however, are essential.

It is often specially important that the feed consumed by single animals under natural grazing conditions be estimated, and it is with this objective in view that a series of experiments have been conducted at Ruakura. The principles embodied in the methods described by Woodman *et al.* (1937) and Garrigus *et al.* (1936) have been applied. These methods depend on the measurement of fæces output and the calculation of intake using digestibility coefficients obtained in a separate experiment. The accepted method of obtaining the digestibility data is by trial with animals. It is well known that the drawbacks to digestibility trials are the heavy demands made on man-hours, and on experimental equipment and materials. It has, however, recently been demonstrated that a different approach to the problem, employing simplified methods of work, eliminates some of these drawbacks while considerably reducing the others. It has been found (Mitchell 1942, Lancaster 1944, McMeekan 1944) that the fibrous constituents of feeds are highly correlated negatively with the digestibility of the feed, and that with sufficient data regression equations connecting digestibility with lignin or crude fibre may be developed.

It is recognized, however, that digestibility data obtained from pasture samples, whether by analysis and regression equation, or by digestibility trial, are subject to the limitation that the sample taken may not represent the herbage consumed by the grazing animal. This is their most serious deficiency, and, as there seems to be no way of estimating sampling errors of this type, the accuracy of intake data depending on digestibility coefficients obtained as suggested cannot be assessed. In this paper, a method is proposed by which the digestibility of pasture actually consumed by the grazing animal may be estimated from the nitrogen concentration in fæces derived from the pasture.

EXPERIMENTAL DATA

The material presented is based on data from the following digestibility trials:—

(1) Three trials at Lincoln College (Lancaster 1944) marked (a) in column 1 in Table I.

(2) Sixteen trials at Lincoln College and the Animal Research Division's Experimental farm at Kirwee, mid-Canterbury (Bartrum and Ewer) marked (b) in column 1, in Table I.

(3) Thirty-three trials at Ruakura Animal Research Station (unpublished) marked (c) in Table I.

Only data necessary to the present thesis are submitted since full experimental observations from which the data were derived are to be published in connection with studies on nutritive status of pastures and other fodder crops. A brief description of the pastures used is considered desirable.

Some of the pastures used in the Kirwee and Lincoln College trials have already been described (Lancaster and Adams 1944). The material used in the subsequent trials indicated in (2) above, consisted of mixed clovers, perennial ryegrass and short rotation (H.I.) ryegrass. The pastures studied at Ruakura were the perennial ryegrass – white clover – cocksfoot associations typical of the Waikato, and contained varying proportions of paspalum in the summer months. At both Kirwee and Ruakura, digestibility data were obtained for pastures at

varying stages of maturity, and the Ruakura series, trials 87 to 113 in the Table, are particularly useful since they were obtained from an almost continuous series of trials conducted from October 1946 to March 1947. The dominant species in the pastures used in each trial are indicated in column 3 of the Table.

TABLE I.

Explanation of Symbols used in the Table

C = Nitrogen g. excreted in fæces per 100 g. pasture organic matter consumed.
n = Nitrogen content of fæces organic matter (per cent.).
D = Organic matter digestibility obtained by trial with sheep.
D_n = Organic matter digestibility obtained by calculation from $D_n = \frac{100_n - 83}{n}$

Trial No.	Sheep	Pasture Herbage.	C.	n.	D.	D_n.	Means. D.	Means. D_n.	Diff.
7(a)	42	Italian ryegrass	0·83	4·43	81·3	81·3	83·1	81·3	+1·8
	229		0·64	4·41	85·4	81·2			
	250		0·78	4·46	82·6	81·4			
6(a)	N25	Subterranean clover	0·87	5·66	84·7	85·3	84·8	83·9	+0·9
	N79	perennial ryegrass	0·73	5·10	85·7	83·7			
	L116		0·76	4·77	84·0	82·6			
5(b)	L37	Perennial ryegrass	0·80	3·57	77·5	76·8	77·9	76·5	+1·4
(1944)	M31	and mixed clovers	0·75	3·47	78·3	76·1			
6(b)	L262	Short rotation	0·91	3·72	75·6	77·7	76·7	77·8	−1·1
(1944)	L417	ryegrass	0·83	3·74	77·7	77·8			
10(b)	L417	Perennial ryegrass	0·77	4·74	83·7	82·5	83·7	82·5	+1·2
(1944)	M56		0·77	4·70	83·7	82·4			
11(b)	M31	Short rotation	0·74	4·85	84·7	82·9	84·9	82·9	+2·0
(1944)	L37	ryegrass	0·72	4·81	85·1	82·8			
6(b)	L37	Short rotation	1·07	4·66	77·0	82·2	83·1	82·9	+0·2
(1942)	L387	ryegrass	0·82	4·50	81·8	81·6			
	M56		0·66	5·07	87·1	83·6			
	746		0·70	5·22	86·5	84·1			
9(b)	M56	Perennial ryegrass	0·72	4·32	83·2	80·8	81·4	80·6	+0·8
(1942)	L37		0·82	4·32	81·0	80·8			
	M31		0·84	4·22	80·0	80·3			
10(b)	L387	Short rotation	0·94	4·54	79·3	81·7	80·2	81·0	−0·8
(1942)	746	ryegrass	0·87	4·28	79·7	80·6			
	L262		0·80	4·32	81·6	80·8			
10(b)	L37	Perennial ryegrass	0·84	4·38	80·8	81·1	81·1	80·8	+0·3
(1943)	L56		0·80	4·26	81·3	80·5			
11(b)	746	Short rotation	0·88	4·51	80·5	81·6	80·3	81·8	−1·5
(1943)	L387	ryegrass	0·92	4·61	80·0	82·0			
12(b)	M31	Perennial ryegrass	0·98	4·10	76·1	79·8	77·5	80·0	−2·5
(1943)	L37		0·94	4·08	76·8	79·7			
	M56		0·87	4·23	79·5	80·4			
13(b)	L387	Short rotation	1·06	4·77	77·8	82·6	78·7	82·3	−3·6
(1943)	L262	ryegrass	0·98	4·55	78·5	81·8			
	746		0·96	4·70	79·7	82·4			

TABLE 1. Continued

Trial No.	Sheep	Pasture Herbage.	C.	n.	D.	D_n.	Means. D.	Means. D_n.	Diff.
14(b) (1941)	L116	Browntop, perennial	0·90	3·90	76·9	78·7	78·9	77·5	+1·4
	M79	ryegrass, and white	0·67	3·49	80·9	76·2			
		clover							
5(b) (1942)	L387	Perennial ryegrass	0·80	5·22	84·7	84·1	84·3	84·3	—
	746	and mixed clover	0·87	5·17	83·1	84·0			
	M56		0·84	5·34	84·3	84·5			
	L584		0·81	5·41	85·1	84·7			
87(c)	1	Perennial ryegrass,	0·67	4·64	85·5	82·1	82·1	81·3	+0·8
	2	cocksfoot, and	0·82	4·27	80·8	80·6			
	5	white clover	0·88	4·40	79·9	81·1			
88(c)	4	Perennial ryegrass,	1·07	4·42	75·7	81·2	76·6	81·3	−4·7
	7	cocksfoot, and	1·03	4·58	77·1	81·9			
	8	white clover	0·99	4·34	77·1	80·9			
89(c)	2	Cocksfoot, white	0·90	3·89	77·0	78·7	77·6	78·8	−1·2
	7	clover	0·89	3·96	77·6	79·0			
	8		0·86	3·92	78·2	78·8			
90(c)	1	Perennial ryegrass,	0·86	4·32	80·0	80·8	79·7	81·3	−1·6
	4	cocksfoot, and	0·94	4·36	78·4	81·0			
	5	white clover	0·88	4·61	80·8	82·0			
91(c)	2	,, ,, ,,	0·78	3·89	80·0	78·7	80·0	78·5	+1·5
	7		0·77	3·89	80·1	78·7			
	8		0·76	3·81	80·0	78·2			
92(c)	1	,, ,, ,,	0·78	3·86	79·8	78·5	80·7	79·1	+1·6
	4		0·75	3·94	80·9	78·9			
	5		0·76	4·13	81·3	79·9			
93(c)	D	,, ,, ,,	0·86	3·71	76·9	77·6	77·5	77·3	+0·2
	E		0·82	3·68	77·8	77·4			
	F		0·80	3·59	77·8	76·9			
94(c)	A	,, ,, ,,	0·79	3·78	79·1	78·0	78·3	78·5	−0·2
	B		0·89	3·99	77·6	79·2			
	C		0·83	3·82	78·2	78·3			
95(c)	D	,, ,, ,,	0·83	3·52	76·4	76·4	76·9	76·0	+0·9
	E		0·77	3·39	77·2	75·5			
	F		0·79	3·46	77·0	76·0			
96(c)	A	,, ,, ,,	0·90	4·21	78·7	80·3	80·0	81·3	−1·3
	B		0·89	4·55	80·4	81·8			
	C		0·87	4·58	81·0	81·9			
98(c)	2	,, ,, ,,	0·71	3·15	77·6	73·7	78·8	75·1	+3·7
	7		0·70	3·33	79·1	75·1			
	8		0·72	3·52	79·6	76·4			
62(c)	1	,, ,, ,,	0·89	4·10	78·3	79·8	78·3	79·9	−1·6
	2		0·87	4·17	79·1	80·1			
	3		0·92	4·11	77·6	79·8			
79(c)	1	,, ,, ,,	1·09	4·08	80·0	79·7	80·5	79·9	+0·6
	2		1·07	4·37	80·9	81·0			
	3		0·85	4·14	79·7	80·0			
	5		0·79	4·00	81·2	79·2			

TABLE 1. Continued

Trial No.	Sheep	Pasture Herbage.	C.	n.	D.	D_n.	Means. D.	Means. D_n.	Diff.
119(c)	A	,, ,, ,,	0·79	4·13	80·9	79·9	80·2	80·5	−0·3
	D		0·80	4·26	81·2	80·5			
	F		0·94	4·36	78·5	81·0			
9(a)	28	Mixed pasture	0·80	3·07	73·9	73·0	73·5	73·1	+0·4
	74		0·84	3·10	73·0	73·2			
1(b)	M56	Perennial ryegrass	0·78	3·17	75·3	73·8	73·8	72·2	+1·6
(1943)	L37		0·85	2·90	70·5	71·4			
	M31		0·70	2·90	75·7	71·4			
2(b)	L387	Short rotation	0·82	2·82	71·1	70·6	70·6	70·8	−0·2
(1943)	746	ryegrass	0·85	2·85	70·1	70·9			
15(b)	L116	Subterranean clover	0·80	2·50	68·1	66·8	68·9	68·0	+0·9
(1941)	M79	and perennial ryegrass	0·82	2·69	69·6	69·2			
3(b)	M56	Mixed pasture	0·79	2·53	68·6	67·2	67·1	66·8	+0·3
(1942)	L262		0·84	2·43	65·6	65.9			
	L387		0·83	2·53	67·0	67·2			
97(c)	1	Perennial ryegrass,	0·75	2·74	72·6	69·7	73·8	70·1	+3·7
	4	white clover, and	0·72	2·83	74·4	70·7			
	5	cocksfoot	0·71	2·77	74·4	70·0			
99(c)	1	,, ,, ,,	0·73	2·86	74·4	71·0	74·5	72·4	+2·1
	4		0·76	3·01	74·8	72·4			
	5		0·82	3·17	74·3	73·8			
100(c)	2	,, ,, ,,	0·74	2·77	73·2	70·0	74·4	70·6	+3·8
	7		0·74	2·85	74·2	70·9			
	8		0·69	2·85	75·7	70·9			
101(c)	1	,, ,, ,,	0·78	3·04	74·2	72·7	74·6	72·5	+2·1
	4		0·76	2·98	74·6	72·2			
	5		0·75	3·02	75·0	72·5			
102(c)	2	,, ,, ,,	0·67	2·34	71·4	64·5	72·0	66·9	+5·1
	7		0·71	2·54	72·0	67·3			
	8		0·73	2·66	72·5	68·8			
103(c)	1	,, ,, ,,	0·74	2·78	73·5	70·2	73·7	70·4	+3·3
	4		0·78	2·96	73·5	72·0			
	5		0·69	2·67	74·2	68·9			
104(c)	2	,, ,, ,,	0·64	2·02	68·3	58·9	68·6	60·1	+8·7
	7		0·65	2·06	68·6	59·7			
	8		0·67	2·16	69·0	61·6			
105(c)	2	Perennial ryegrass,	0·72	2·77	73·9	70·0	72·1	70·7	+1·4
	7	white clover, cocks-	0·78	2·66	70·5	68·8			
	8	foot, and paspalum	0·83	2·83	70·6	70·7			
	c		0·83	3·09	73·2	73·1			
106(c)	1	,, ,, ,,	0·77	2·74	71·9	69·7	69·8	68·3	+1·5
	4		0·74	2·54	70·7	67·3			
	5		0·86	2·59	66·9	68·0			

TABLE 1. Continued

Trial No.	Sheep	Pasture Herbage.	C.	n.	D.	D_n.	Means. D.	Means. D_n.	Diff.
107(c)	2	,, ,, ,,	0·93	2·56	63·0	67·6	62·9	67·5	−4·6
	7		0·96	2·52	62·0	66·9			
	8		0·94	2·59	63·7	68·0			
108(c)	1	,, ,, ,,	0·93	2·11	56·0	60·7	56·3	63·4	−7·1
	4		1·09	2·45	55·5	66·1			
	5		0·96	2·26	57·3	63·3			
109(c)	2	,, ,, ,,	0·93	2·63	64·7	68·4	65·2	68·9	−3·7
	7		0·91	2·67	65·8	68·9			
	8		0·94	2·70	65·2	69·3			
110(c)	1	Perennial ryegrass	1·02	2·32	56·0	64·2	57·1	62·2	−5·1
	4	and white clover	0·92	2·16	57·3	61·6			
	5		0·89	2·11	57·9	60·7			
111(c)	7	As in trials 97 *et*	0·96	2·59	63·1	68·0	62·9	69·5	−6·6
	8	*seq.* but drying out	1·07	2·85	62·6	70·9			
112(c)	1	,, ,, ,,	1·01	2·24	55·2	63·0	55·8	62·7	−6·9
	4		0·97	2·21	56·3	62·4			
113(c)	2	As in trial 111 but	0·92	1·82	49·3	54·4	49·6	54·7	−5·1
	5	dry and stalky	0·92	1·84	49·8	54·9			
114(c)	A	Pure paspalum	0·78	2·38	67·4	65·1	66·8	65·1	+1·7
	E		0·80	2·37	66·2	65·0			
64(c)	1	Perennial ryegrass,	0·64	2·59	75·2	68·0	76·2	70·4	+5·8
	2	white clover, and	0·68	3·15	78·4	73·7			
	3	cocksfoot	0·68	2·72	75·0	69·5			

DISCUSSION

The data essential to the discussion are presented in the table. Whilst the results of the digestibility trials were under consideration, it was noticed that the amount of nitrogen excreted in fæces per 100 g. of pasture dry-matter consumed was remarkably constant, the average being 0.72 g. A preliminary note to this effect has already been published (Lancaster 1947). It is outside the scope of this paper to discuss the fundamental implications of this observation, but its application to the calculation of digestibility is described.

Interest at Ruakura lies mainly in the intake of pasture organic matter rather than dry matter since the latter figure is sometimes of doubful value owing to contamination of pasture with soil or dust. Accordingly, data are presented in terms of organic matter, to provide for the calculation of "organic matter digestibility." The constant referred to above—the average amount of nitrogen excreted per 100 g. of pasture organic matter consumed—was 0.83 ± 0.102 g.

The calculation of organic matter digestibility is based on the hypothesis that the nitrogen excreted in sheep fæces per 100 g. of organic matter consumed is a constant 0.83 g. for the types of pasture under consideration. Suppose that analysis showed a sample of sheep fæces to contain n per cent of nitrogen calculated on the organic matter basis. By hypothesis 0.83 g. of fæces nitrogen was excreted per 100 g. of pasture organic matter consumed. Therefore, n g. of nitrogen was excreted per $\frac{100\,n}{0.83}$ g. of pasture organic matter consumed. But n grams

of nitrogen was shown to be contained in 100 g. of fæces organic matter. Therefore, 100 g. of fæces organic matter was associated with or derived from $\frac{100\,n}{0.83}$ g. of pasture organic matter.

The per cent digestibility of organic matter is therefore:—

$$D = 100\left[\left(\frac{100\,n}{0.83} - 100\right) \div \frac{100\,n}{0.83}\right] = \frac{100\,n - 83}{n}$$

In a preliminary paper discussing the use of this equation (Lancaster 1947) the calculation of dry matter digestibility was presented, and nitrogen figures were quoted in terms of protein. This explains the difference between the constant 4.5 used on that occasion, and the one employed in this paper.

The value of the constant 4.5 (.72 calculated as nitrogen) has been confirmed by Gallup and Briggs (1948). These authors have shown from data of Morrison (1936) and Mitchell (1942) that "for roughages as a class, containing from 8 to 24 per cent protein, the constant ranged from 0.67 to 0.76 g. N per 100 g. dry matter consumed."

The results of the application of the above equation to fæces nitrogen data from all pasture digestiblity trials conducted with sheep at Lincoln College and Ruakura are tabulated in Table I.

Inspection of the figures under columns D and D_n indicates that the fæces nitrogen method of estimating digestibility yields results which in many cases are only slightly different from those obtained by the orthodox digestibility trial. If the mean figures obtained by the two methods in each trial are considered, it is found that in three-quarters of the trials on pasture having digestibility in the range 70 - 85 per cent, the difference between results due to the two methods was $\pm$ 2 per cent or less. With pastures having digestibilities in the range 50 - 69 per cent, however, the difference is $\pm$ 2 per cent or less in only one-third of trials.

Repeatability is an important consideration in any experimental technique, and on this count, the fæces nitrogen method is somewhat superior to the conventional method. This is shown by an analysis of variance of the data which yielded a within-trial variance of D of 2.61 digestibility units compared with a within-trial variance of D_n of 1.22 units.

That the prediction equation operates much more satisfactorily in the high digestibility range is readily explainable. The variable n in the equation occurs in the denominator, so that the higher the value of n, which increases with digestibility, the smaller would be the error in the calculation of digestibility, due to deviations from the hypothesis upon which the equation is based. Furthermore, there is a pronounced asymetric distribution of the digestibility data toward the high digestibility end of the range, and it is only to be expected that a formula derived from all the data would favour the digestibility range in which most of the data occur.

In view of the known effects of pasture constituents, e.g. protein (Mitchell 1943, Blaxter and Mitchell 1948) on the excretion of metabolic nitrogen in the fæces, it would be surprising if the constant 0.83 used in the present formula yielded accurate results over the whole range of pastures studied. It is obvious, of course, that it would be possible to fit a curve more accurately to the available data than that which has been used, but it is considered desirable to await more information on the low digestibility material before this is attempted.

In the meantime, the validity of employing the fæces nitrogen method of estimating digestibility by grazing sheep must be considered. In applying to grazing sheep digestibility coefficients obtained by the conventional method with stalled sheep, two assumptions are made: (a) that the material gathered for feeding to stalled sheep is a representative sample of that selected by the grazing animal; (b) that the sheep under conditions of free grazing would digest the material to the same extent as it does in the stalls. There are obvious technical difficulties in testing the validity of either assumption, but field grazing observations suggest that it is most unlikely that (a) is justified. In applying the fæces nitrogen prediction equation to grazing sheep, neither of these assumptions is necessary, but it must be postulated that the constant 0.83 still holds with the grazing animal. The validity of this is as difficult to test as the first two assumptions.

It is suggested, therefore, that apart from the errors demonstrated by the data in Table I, no more errors would be introduced into the estimate of digestibility of pasture herbage consumed by a grazing animal than might be introduced by applying the results of orthodox digestibility trials to the same purpose. The elimination of the pasture sampling error is a very considerable advantage in favour of the fæces nitrogen method. This advantage is more pronounced when dealing with the low digestibility pastures normally encountered in the summer period. The mixed nature of these in consequence of the presence of seed heads, and other stalky material, often associated with young growth, makes sampling a difficult operation. It is possible, therefore, that although the fæces nitrogen method may result in considerable apparent errors in estimates of digestibility of this type of pasture, yet the method may be more accurate than any requiring pasture sampling. The simplicity of the nitrogen method is obvious, and can be fully exploited in a technique of measuring the amount of herbage consumed by the grazing animal, for which the collection of fæces is a prerequisite.

ACKNOWLEDGMENTS

The author wishes to thank Mr. P. J. Armstrong of the Biometrics Section, Department of Scientific and Industrial Research, for an analysis of variance of the data presented in this paper. Thanks are also due to Miss M. P. Bartrum of Ruakura Animal Research Station for permission to quote from her material which will shortly be published.

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INCREASES IN THE MOLYBDENUM CONTENT OF PASTURE ON AN ACID PEAT SOIL FROM TOPDRESSING WITH AMMONIUM MOLYBDATE

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(*Received for publication, 6th October, 1948*)

Summary

Ammonium molybdate as a topdressing on an acid peat soil caused a high and persistent increase in the molybdenum content of the pasture. The increase varied with the rate of topdressing and was still present after two years. The rates of application ranged from 5 oz. to 14 lb. per acre.

Introduction

In the course of experimental work on the etiology of "peat scours" in New Zealand it was necessary to make some observations on the increases in molybdenum content of herbage that could be brought about by the application of ammonium molybdate as a fertilizer. The work was carried out at Waitakaruru on acid peat soils which differ from most other peat soils in New Zealand in growing pasture which is comparatively low in molybdenum content.

An acid soil medium is generally believed to be unfavourable to the uptake of molybdenum by plants. The results obtained on the particular peat soils referred to show a high uptake of molybdenum and may therefore be of some interest.

Experimental Method

Preliminary topdressing trials were carried out on a series of small plots, each 1/200 acre in area, and the findings from these plots were applied to the topdressing of a 17-acre paddock. Ten plots in two separated groups of five were used for the plot trials and the rates of topdressing were duplicated in each group. Ammonium molybdate was employed as the topdressing and the rates were as follows:—

Plot No.	1 & 6	2 & 7	3 & 8	4 & 9	5 & 10
lb./acre.	¾	1¾	3½	7	14

The molybdate was finely ground and the appropriate quantity for each plot was mixed with agricultural lime equivalent to 2 cwt. per acre to facilitate application.

The plots were topdressed on 1st May 1946 and were sampled at irregular intervals thereafter on the dates shown; a control area adjacent to each group of plots was sampled at the same times. Table I shows the average results for each rate of topdressing.

In the paddock scale topdressing a 17-acre paddock near the plots was topdressed on 28th May 1947 using 6 lb. ammonium molybdate for 17 acres (approximately 5 oz. per acre) plus 2 cwt. agricultural lime per acre.

The method of determination of molybdenum in the pasture was that described by Perrin (1).

Results

The quantity of molybdenum found in samples of the previously topdressed pastures is set out in Table I.

TABLE I. MOLYBDENUM CONTENTS OF PASTURES TOPDRESSED WITH AMMONIUM MOLYBDATE
(Parts per million Mo. in Dry Matter)

Plot No.	Ammonium Molybdate topdressing.	18/6/46	16/7/46	27/8/46	15/10/46	17/12/46	22/4/47	15/7/47	29/9/47	19/11/47	16/7/48	pH 28/4/48
	lb. per acre on 1/5/46											
Controls	0	2·8		3·2	2·6	1·4	3·1	3·7	3·5	2·3	3·0	
1 and 6	¾	100	100	58	33	18	25	27	22	11·5	11	5·4
2 and 7	1¾	178	146	119	76	33	56	59	48	28	27	5·2
3 and 8	3½	344	339	185	111	68	72	101	97	53	58	5·0
4 and 9	7	486	475	324	235	91	108	188	160	87	92	5·4
5 and 10	14	615	671	521	340	166	252	300	254	165	137	5·2
Paddock 46 B	5 oz. per acre on 28/5/47				2·0		1·4	40	28	26	27	4·7

Discussion

The results show that when ammonium molybdate is applied as a topdressing the molybdenum is readily taken up by the pasture plants and the effect persists for a long period. As little as 5 oz. ammonium molybdate per acre produced a big increase and higher amounts produced roughly proportionally bigger increases in the molybdenum content of the pasture. The effect of 5 oz. per acre was still pronounced eleven months after the topdressing was applied, i.e. up to the latest date of sampling the pasture. For the higher levels of topdressing the observations cover nearly two years and a marked effect was demonstrated for this period for all rates of topdressing from $\frac{3}{4}$ lb. per acre up to 14 lb. per acre.

The small amount of lime used in applying the molybdate had no appreciable effect on the pH of the soils. These were acid on 28th April 1948 (see Table I) and the molybdenum content of the pastures was considerably higher than that of the controls.

The pasture growing on the plots and the paddock contained about 70 per cent of Yorkshire fog (*Holcus lanatus*). This may have contributed in some measure to the high uptake of molybdenum since it has been shown on "teart" pastures that fog has a higher content of molybdenum than other pasture species in the same sward.

Certain minimal levels of molybdenum are required in the soil to promote symbiotic nitrogen fixation (2). High levels of molybdenum are, however, not desirable in pasture. A scouring disease on the "teart" lands of Somerset, England, has been shown to be due to the high level of molybdenum in the pasture (3) and it appears probable that moderately high pasture molybdenum together with low pasture copper is the cause of peat scours in New Zealand. The particular interest of the results reported here is that the acid condition of the peat soils does not prevent plants growing on them from absorbing molybdenum with great readiness. In contrast, one of the methods to reduce molybdenum and thus reduce "teart" is to employ acid fertilizers and avoid lime so as to keep the soil pH low (4). The use of small dressings of ammonium molybdate to increase the molybdenum content of pastures provides a means of studying the part played by molybdenum in the etiology of peat scours in New Zealand. Such experiments are, in fact, proceeding.

Acknowledgment

The authors' thanks are due to Mr. A. C. S. Wright of the Soil Survey Division, Department of Scientific and Industrial Research, for carrying out the pH determinations on the soil samples.

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THE CONTROL OF COPPER DEFICIENCY IN LAMBS IN NEW ZEALAND

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(*Received for publication, 22nd October, 1948*)

Summary

On peat soils in New Zealand copper deficiency symptoms amongst ovines, which are of practical significance in sheep farming, are shown only by lambs which develop enzootic ataxia. This disease is an uncomplicated copper deficiency.

If regular supplies of copper are given to the ewe, either by topdressing the pasture or by drenching, complete control of the disease in lambs is established. The different drenching procedures found effective included three drenches each of 2.5 g. copper sulphate to the ewes in the mid-gestation period, and drenching of ewes started seven weeks before lambing commenced.

Twice weekly drenching of lambs with small amounts of copper, commenced shortly after birth, also prevented any cases of enzootic ataxia.

Symptoms do not develop in lambs born the first year after normal ewes are brought on to copper-deficient areas, but a high proportion of lambs of the second and subsequent years are affected. Control of the disease by this method would require the introduction of fresh ewes each year.

The most satisfactory method of control is to topdress the copper-deficient pastures with copper-containing fertilizers.

INTRODUCTION

IN previous papers (1, 2) the occurrence of copper deficiency on reclaimed peat lands in New Zealand was reported and a description was given of a disease which exists amongst lambs born on these areas. The symptoms of this disease are similar to those of the milder forms of swayback in England (3) and of enzootic ataxia in Australia (4). Lambs up to about four months of age may develop an incoordination or ataxia of the limbs which is permanent and is due to regional demyelination of the nervous system. No lambs which have reached the weaning stage without displaying symptoms of the disease have been observed to develop them later on, and these unaffected weaners can be reared to maturity in a normal manner whilst still on the copper-deficient country.

The disease in New Zealand is preventable, as in Australia and England, by supplying copper to the mother before lambing occurs. The problem of control, therefore, is the problem of supplying sufficient copper to the mother in sufficient time to enable her to produce and rear a lamb that will be normal at weaning. It has already been shown that effective control is achieved by regular drenching of ewes with copper sulphate at weekly intervals throughout the gestation period, or by running the ewes on land topdressed with copper sulphate or other compounds of copper. In this paper the results of some further experiments on control are given.

EXPERIMENTAL METHODS

The methods of investigation were concerned with the following aspects:—

(1) Examination of lambs from ewes transferred from areas not deficient in copper.

(2) Effect of supplying copper to ewes for different periods before lambing.

(3) Effect of supplying copper to lambs from birth onwards.

Lambs from Ewes transferred from Areas Not Deficient in Copper

A mob of Romney 2-tooth ewes reared on an area adequately supplied with copper was brought on to the copper-deficient country at Waitakaruru to measure the time taken before such ewes would produce a significant number of ataxic lambs. For the first season the mob was run as one group on copper-deficient pasture; for the second season the balance remaining after culling was divided into two groups of unequal size, the smaller of which was kept on copper-deficient pasture and the larger on pasture enriched in copper by topdressing with copper salts. The copper-deficient group was unaltered in the third season but the group on topdressed pasture was added to in the third and fourth seasons by inclusion of some of its own female progeny. Table I shows the number of lambs tailed and weaned in these groups for four successive seasons. The copper-content of the livers of some new-born lambs in each group is also given, the number in brackets being the number of livers analysed to give the average figure stated.

TABLE I. WEANING PERCENTAGES OF LAMBS FROM EWES TRANSFERRED FROM AREAS NOT DEFICIENT IN COPPER

Year.	Untreated Area.				Topdressed Area.			
	No. of Ewes.	Cu in Liver of Lambs. *(p.p.m.)	Lambs Tailed. (per cent.)	Lambs Weaned. (per cent.)	No. of Ewes.	Cu in Liver of Lambs. *(p.p.m.)	Lambs Tailed. (per cent.)	Lambs Weaned. (per cent.)
1943-4	270	79 (1)	67	65				
1944-5	93	7·5 (2)	84	59	148	204 (4)	105	105
1945-6	93	7·9 (15)	110	56	159	258 (3)	107	107
1946-7					181	219 (8)	117	111

* Calculated on a dry-matter basis.

The poor tailing and weaning percentages in 1943-44 were probably due to the facts that the sheep were 2-tooths and that they had been brought from a considerable distance on to different country just prior to the rams going out. There were no clinical cases of ataxia seen in this year. In 1944-45 and 1945-46 the discrepancy between the numbers of lambs tailed and those weaned in the group on the untreated area was due to deaths of lambs showing symptoms of ataxia, 23 such cases died in 1944-45 and there were at least 12 cases of ataxia in lambs not tailed. In 1945-46, 50 tailed lambs died with ataxia and there were at least 6 cases in lambs which were not tailed. In the group on pasture topdressed with copper salts, no discrepancy existed between lambs tailed and lambs weaned and no cases of ataxia were recorded in this group in any of the three seasons they were under observation.

It is apparent that in the second season after ewes were brought to the copper-deficient land, a significant number of cases of ataxia occurred in their progeny. In the third season, the number of cases was

still higher and had reached 50 per cent of the lambs tailed. On this area, control of ataxia by bringing in fresh ewes would, to be effective, require fresh ewes each season.

Ewes Supplied with Copper before Lambing

In Table II are shown the results of a series of experiments in several successive years. The number of lambs weaned is taken as the measure of the effectiveness of treatment, and low weaning percentages were always associated with a high occurrence of ataxia in lambs before or after tailing.

From control groups in the three seasons 1943-44 to 1945-46 only 22, 34 and 38 per cent of lambs were weaned and in a small group in 1946-47 no lambs were weaned.

The groups in which copper was supplied to the ewes or to lambs are discussed in succeeding paragraphs. The weaning percentages were much higher and most of the lambs tailed were reared in a healthy state to the weaning stage. There were no clinical cases of ataxia in the lambs from any of the groups to which copper was supplied.

Ewes on Topdressed Areas:

In the four groups shown in Table II, ewes were placed on the topdressed areas during February of the year to which the records apply. The ewes had all been reared and had lived for some time on copper-deficient country and were of similar origin to the animals in the control groups for the same year. Clearly the provision of the extra copper in the topdressed pastures from tupping onwards achieved complete control of ataxia in lambs as evidenced by absence of clinical cases, high weaning percentages, and normal copper content of lambs' livers.

Reference should also be made to Table I, as the data for sheep on topdressed areas shows the effectiveness of keeping ewes continuously on pasture enriched by topdressing with copper salts.

Drenching Ewes:

Several drenching programmes were adopted in different seasons with the objects of determining the effectiveness in prevention of ataxia of regular drenching between tupping and lambing, the minimum number of drenches that would prove effective, and the minimum period before lambing commenced that drenching must be started.

The first three groups (1, 2 and 3) shown in Table II under the heading of "Ewes drenched" received sixteen drenches each of 1.5 g. $CuSO_4.5H_2O$ at approximately weekly intervals from the time tupping commenced. This provided complete control of ataxia in the lambs and adequate supplies of copper were present in livers of ewes and of young lambs.

In the fourth group, five drenches of 1.5 g. $CuSO_4.5H_2O$ administered to the ewes between 6th May and 10th July, 1944, completely prevented ataxia in their lambs. Lambing commenced on 29th August, 1944, and drenching therefore commenced about sixteen weeks before the first lamb was born and was completed seven weeks before this event. In this and other large groups of ewes there were smaller tailing and weaning percentages than in the small groups of ewes. The difference was due to accidental drowning losses of normal lambs in open drains in the large paddocks available for the big groups. In the paddocks used for small groups the open drains were fenced out.

TABLE II. CONTROL OF ENZOOTIC ATAXIA BY COPPER TREATMENT OF EWES OR LAMBS

Year.	Treatment.	When Commenced.		No. of Ewes.	Liver Cu. p.p.m. on dry liver.		Lambs.	
		Date.	Weeks before lambing started.		Ewes.	Lambs. new-born.	Per cent. Tailed.	Per cent. Weaned.
1943-44	None (Controls)			174	13·9 (2)	10·1 (2)	54	22
1944-45				352			59	34
1945-46				138	6·4 (4)	8·1 (62)	56	38
1946-47				10			nil	nil
	Grazed on topdressed area.							
1943-44	1 Topdressed 20 lb. super. per acre.	At tupping		115		576 (10)	102	102
1944-45	2 Topdressed 10 lb. copper sulphate per acre.	,,		49	357	147 (5)	100	92
1946-47	3 Topdressed 10 lb. copper sulphate per acre.	,,		39		209 (2)	128	128
1945-46	4 Topdressed 5 lb. copper sulphate per acre.	,,		923		306 (13)	102	102
	Ewes Drenched.							
1943-44	1 16 drenches 1½ g. copper sulphate at 7 day intervals.	At tupping		113	320 (2)	265 (10)	89	88
1944-45	2 16 drenches 1½ g. copper sulphate at 7 day intervals.	,,		41	98 (2)	159 (4)	122	119
1945-46	3 16 drenches 1½ g. copper sulphate at 7 day intervals.	,,		42	355 (4)	393 (3)	126	119
1944-45	4 5 drenches 1½ g. copper sulphate at 14 day intervals.	6.5.44	16	337			89	89
1944-45	5 3 drenches 2½ g. copper sulphate at 14 day intervals.	8.5.44	16	49		170 (4)	98	88
1946-47	6 16 drenches 1½ g. copper sulphate, 2 drenches per week.	29.6.46	8	18		209 (2)	106	100
1946-47	7 16 drenches 1½ g. copper sulphate, 2 drenches per week.	12.7.46	6	33		111 (4)	124	124
1946-47	8 16 drenches 1½ g. copper sulphate, 2 drenches per week.	25.7.46	4	27		112 (4)	122	122
	Ewes not treated.							
1944-45	Lambs drenched twice weekly from birth.			39	47 (2)	17·4 (5) at birth 264 (2) at weaning	113	110
1945-46	Lambs drenched twice weekly from birth.			47	33 (3)	7·2 (2) at birth	110	109

Three drenches each of 2.5 g. $CuSO_4.5H_2O$ given to the fifth group of ewes between 8th May and 12th June, 1944, also completely controlled ataxia in their lambs. Lambing in this group commenced on 31st August, 1944, and drenching was therefore started about sixteen weeks and was completed eleven weeks before lambing began.

In groups 6, 7 and 8 it was planned to determine the effect of drenching commenced 8, 6 and 4 weeks before lambing began. In group 6 this was carried out but through an oversight drenching was continued in group 7 for one week and in group 8 for three weeks after the commencement of lambing. Approximately 70 per cent of lambs in groups 7 and 8 were not born until after drenching was complete; therefore, for the majority of the animals of these two groups the experiment actually tested the effect of drenching commenced seven weeks before lambing. Each ewe was given two drenches of 1.5 g. $CuSO_4.5H_2O$ per week until a total of sixteen drenches was administered. The total copper supplied was therefore the same as for groups 1, 2 and 3 but the period of supply was shortened to the eight weeks and seven weeks just before lambing. This copper supply completely controlled ataxia in the lambs.

Lambs supplied with Copper

If ewes were untreated but lambs were drenched from birth, the development of ataxia was averted, as is shown by the results of experiments carried out in 1944-45 and 1945-46. In these experiments the ewes were run on copper-deficient country and received no treatment; all lambs, however, were drenched regularly with copper sulphate solutions from shortly after birth until they were about four months old. Each dose for lambs contained 35 mg. $CuSO_4.5H_2O$ (approximately 9 mg. copper) in 0.5 oz. water, and drenching was carried out each Monday and Friday in every week after the first lamb was born. No lamb was therefore more than three days' old before drenching commenced and each lamb received regular treatment twice every week after birth. The low copper content of livers of lambs which died at birth shows that copper supplies during gestation had been small. The low liver copper of ewes killed after weaning the lambs shows that ewes did not obtain any appreciable supply of copper, while the livers of two lambs slaughtered after weaning in the 1944-45 season showed that the lambs received an adequate supply of copper in their drenches.

Discussion

The experimental work described in this paper shows that enzootic ataxia in lambs in New Zealand can be controlled completely and readily by supplying copper to the ewes before lambing or to the lambs after birth. The copper-deficient ewe readily absorbs copper from orally administered sources and efficiently makes it available to the fetal lamb; the new-born copper-deficient lamb to which copper is supplied orally, grows and develops normally. Provision of copper either during antenatal life or early in post-natal life is effective in preventing the occurrence of enzootic ataxia. The effectiveness of post-natal administration of copper would suggest that in the New Zealand disease the final stages of the pathological processes which give rise to ataxia occur after birth, possibly during the final development of the nervous system which is thought to be a post-natal phenomenon.

There is no evidence of any factor other than a simple copper deficiency being responsible for enzootic ataxia in New Zealand. It occurs, as has been shown previously (2) on copper deficient pastures, and the livers of affected lambs and their mothers are grossly deficient in copper. Its prevention is a result of supplying copper. The disease in lambs, therefore, is similar to enzootic ataxia in Australia and is not complicated by factors other than a dietary deficiency of copper, as is apparently the case with swayback in Great Britain (5).

Consideration of the results obtained in the present work from the angle of control of enzootic ataxia under practical farming conditions shows that none of the drenching procedures tested possesses any advantage over topdressing the copper-deficient pastures with fertilizers containing copper. This procedure gave complete control when deficient ewes were put on to such topdressed pastures at tupping time or when ewes were kept on topdressed pasture continuously for four years. Drenching or provision of copper by some other means might have to be resorted to in cases where topdressing is impracticable because of difficulties of terrain or other factors. The minimum number of drenches tried was three, each of 2.5 g. copper sulphate at fortnightly intervals, completed eleven weeks before lambing commenced. This might be considered as one resort in the absence of more convenient measures. The efficacy of licks in supplying copper could not be tried because the sheep in the experiment would not take licks.

While this paper is primarily concerned with the results of experiments on the control of copper-deficiency symptoms in lambs it may be of interest to comment upon the condition of older sheep. Manifestations of copper deficiency which are of practical significance to sheep farming have so far been observed only in lambs prior to weaning. No animals which have reached the weaning stage without developing symptoms of ataxia have been observed to become affected thereafter. Wether lambs can be reared and sold in prime fat condition and ewes can be used for many years for breeding and show no apparent abnormality in general thrift or fertility. In some years, some of the ewes become anæmic but in other years anæmia does not occur.

Observations on the wool production of copper-deficient ewes have been inconclusive; there have been indications that fleece weight might be somewhat improved by supplying copper but results in successive years have not been consistent. There is, however, no abnormality of the wool comparable to the steely wool observed in the Australian Merino (4) (6). The Romney breed is the one employed on the copper-deficient area in New Zealand and this difference may be a breed difference or, on the other hand it may be a result of a difference in the degree of copper deficiency. In this connection, the pastures contain on the average about three parts per million of copper in the dry matter which would provide each ewe with approximately 4 to 6 mg. copper per day in its fodder; this is appreciably higher than the 2 to 4 mg. per day calculated by Marston and Lee (7) to be available to sheep on coast disease areas of South Australia. The observations made above on adult sheep will be amplified and discussed more fully in a later paper. They refer, as does the work on lambs, to only one copper-deficient area, that on the peat deposits of Waitakaruru in the Hauraki Plains. This is the only area on which sheep have been carried regularly; on other copper-deficient peat areas any ewes which are employed for breeding are replaced regularly. Some areas of leached sandy soils

even more deficient in copper than the peat lands have recently been discovered, and if sheep farming is carried on in these areas without provision of copper more acute copper deficiency might occur.

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REVIEW:

ADVANCES IN GRASSLAND HUSBANDRY AND FODDER PRODUCTION.

BULLETIN NO. 38 OF THE IMPERIAL BUREAU OF PASTURES AND FIELD CROPS.

This is the second symposium of the series in which the Imperial Bureau is bringing together miscellaneous papers and reviews from the world literature covering the central theme of fodder production and pasture utilization.

As would be expected in a symposium covering such a wide field, it is not easy to pick up the connection between the various papers from a cursory study. This is probably even more apparent to readers in countries where the climatic conditions are fairly uniform, and agriculture conforms to a more or less even type. However, the New Zealand reader will find much of interest and value in the comparison of the many methods discussed with those obtaining throughout the different parts of this country with its great variability of climate and structure.

The publication includes four main papers and a series of brief reviews and reports. A paper from Southern Rhodesia gives a review of climatic conditions and a natural vegetation survey together with details of the current research programme for veld and pasture management studies. In this the central theme hinges around the maintenance of the *natural* grasses of the veld by the combination of cattle grazing and controlled burning or mowing, as well as finding the optimum ratio of trees and browsing shrubs to grassland.

What is described as an exploratory article from the U.S.S.R. discusses the variability of natural formations of grass and legume species and the relationship with geological activity, whilst in marked contrast Professor Ashby of Oxford University, in another paper, deals with the economics of ley farming in England and the use of the latest bred pasture strains in the changing conditions of British agriculture. Especially stressed in this respect is the greater use of mechanical power in the arable districts, and also the intensification of farming generally following the higher prices being obtained for agricultural products in Great Britain.

Of considerable interest is a paper from Switzerland, in which are set out the war-time agricultural changes in that country and the impact of its neutrality and policy of self-sufficiency on the agricultural research outlooks. By the extension of co-operative research and advisory services, improvement in the forage crop programme was implemented and thus, although there was a large decline in stock units carried, the drop was much smaller than the reduction in area available for fodder production. The basis of this improvement programme was the sowing of higher producing pastures, the use of all animal and crop residues for animal feeding, and a reduction in conservation losses (hay making, ensilage and artificial drying).

The remainder of the bulletin consists of short reports and reviews covering a wide variety of subjects from several research stations on a world-wide geographical coverage.

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